PLANT PHYSIOLOGY

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ERRATA, VOLUME 3

Page 30, citation 16, following name, insert Über die Verteilung des osmotischen.
Page 36, line 12, for $.4HO_2O$ read $.4H_2O$.
Page 41, last line, for fraction read fractionation.
Page 62, first line of footnote, for $CaCo_3$ read $CaCO_3$.
Page 79, text lines 16 and 18, for xanthroproteic read xanthoproteic.
Page 113, line 7, for homogenity read homogeneity.
Page 169, line 5, for heighth read height.
Page 174, line 13, for flash read flask.
Page 183, line 8, for landwitschaftlichen read landwirtschaftlichen.
Front cover, July no., title 5, for hardness read hardiness.
Page 239, control solution, for $Al_2(So_4)_3$ and $CuSo_4$, read $Al_2(SO_4)_3$ and $CuSO_4$.
Page 309, text line 7, for findinge read findings.

Vol. 3 No. 1

PLANT PHYSIOLOGY

JANUARY, 1928

STUDIES UPON WHEAT GROWN UNDER CONSTANT CONDITIONS—I

H. L. van de Sande-Bakhuyzen

Introduction

From an economic point of view it is very important to find a method of making a trustworthy forecast of the yield of a crop. It has been found that in several agricultural crop plants there is a marked correlation between cool, rainy weather at about the time of flowering and a high crop yield. It has, moreover, been found advantageous to irrigate cereals at this period. The purpose of the present investigation is to determine whether there is a physiological basis for the contention that the flowering stage in plants, especially in wheat, is a critical period in the life cycle. As wheat is one of the most important agricultural plants, in which, moreover, the Food Research Institute of Stanford University has been especially interested, our experiments were carried out with this plant. An early-maturing spring wheat variety, Hard Federation, was chosen. The seed was obtained through the kindness of Dr. V. H. Florell, of the U. S. Department of Agriculture, from the Agricultural Experiment Station at Davis, California, and was grown from a pure line.

By the term critical period is meant a period in the life cycle of the plant during which the correlation between external conditions, *i.e.*, rainfall or temperature, and the final yield of the crop is highest. Upon the internal conditions of the plant, *i.e.*, the physiological state of its cells, external conditions exert their influence, thereby changing the physiological activities of the cells, *i.e.*, the development of the grain. We should, therefore, study the internal conditions of the plant throughout its life cycle and, especially, during the period of flowering. If it were possible to detect one weak link in the chain of internal physiological processes accompanying the transition from the vegetative to the reproductive stage (development of

new sporophyte), it would be probable that such a link might act as a limiting factor if the external conditions were such as to attenuate it. In order to study the internal processes which normally go on in a plant it is necessary to grow plants under constant and optimal conditions.

In recent years several investigators have grown plants under constant controlled conditions, but very little is known as yet concerning the internal processes throughout the life cycle. For several reasons the importance of external conditions upon the vital phenomena in plants has been overemphasized (**2**) and even now the effect of one set of constant conditions is usually compared with the effect of another without thoroughly studying the behavior of the plant under any given set of conditions. After this behavior is known exactly, the study of the processes at some other level may be begun, and thus the effect of external conditions ascertained. In our experiments the temperature, illumination, humidity of the air, moisture and salt content of the substratum (sand or solution) were kept as constant as possible.

Experiment room and material

The experiments were carried out in the basement of the Botany Building, Stanford University, in a room 7.8 m. long, 2 m. wide and 2.5 m. high. From the ceiling were suspended fifteen 300-watt Mazda C. lamps, evenly distributed throughout the length of the room. Directly under the lamps, fifteen chimneys, 12 cm. in diameter, connected with a tapering air tunnel about 50 x 25 cm. in diameter, placed under a table, protruded through fifteen holes in the table. This tunnel (of which the end within the room was closed) opened into a side room connected with a long corridor, where a large air space was available. In the middle of one of the long walls opening into the corridor, an electric fan, 50 cm. in diameter, sucked the air out of the closed experiment room. The fifteen chimneys afforded the only entrance for fresh air and a current was produced at the rate of 270 meters per minute, which cooled the lamps and gave the necessary ventilation. This amount of air replacement was required in order to maintain a proper temperature, as the lamps produced much heat. The chimneys ended approximately at the height of the top of the crocks in which the plants were grown. The lamps were 1.20 m. above the top of the crocks.

Two Draper thermographs and one Draper hygrograph recorded the temperature and humidity of the air. The latter was checked with a Tycos dry-and-wet-bulb thermometer. From the average A. M. and P. M. readings an average daily reading was found; from these data the mean daily temperature and humidity with probable errors were calculated. Daily fluctuations in the temperature did not occur; the humidity of the air showed only secular fluctuations. If the latter showed a trend to rise

or fall, the values between the end and the beginning of the week did not differ more than 5 per cent. The largest deviation from the mean temperature amounted to $\pm$ 1.2° C., the largest deviation from the mean humidity was $\pm$ 8 per cent. Both maximum deviations were rare, as can be seen from the probable errors, given in the next section. No special devices were used for keeping temperature and humidity of the air constant, as during the experiments the air in the long corridor was very constant in these respects.

During the entire period of the experiments the lights burned 24 hours a day. In order to economize light energy, white cardboard screens suspended from the ceiling surrounded the table; in this way reflection took place in all directions. If the distance between the lamps and the plants is large enough, the light intensity must remain constant independent of the distance. In our case it decreased somewhat less than the first power of the distance. Fifteen lamps of 300 watts each produce $15 \times \frac{4900}{2} = 36750$ lumens. The area between the screens was $25 \times 2.1 = 52.5$ sq. ft., *i.e.* per sq. ft. a quantity of $\frac{36750}{52.5} = 700$ lumens or foot-candles was received. As the lamps were, however, so close to the plants that the reflectors intercepted part of the light, the intensity as measured with a Leeds and Northrup Macbeth Illuminometer gave only a value of 600 foot-candles. After about a month the lamps had lost 20 per cent. of their intensity and were renewed.

In the first experiment the sand in which the wheat was grown was washed with 3 per cent. hydrochloric acid, then with water to neutrality and finally with distilled water. In the second experiment, however, this procedure was omitted as it was too laborious and as the test for chlorine showed that only a very slight amount was present. A three-salt solution with an osmotic pressure of 1 atmosphere was used. Iron was added in the form of tartrate. By weighing the crocks, a soil moisture of about 10 per cent. was maintained throughout the experiment. In the beginning of the experiment every other day, later on every day, the water lost by evaporation and transpiration was restored in the form of distilled water or nutrient solution.

After a selection of the seed by hand and by a screen, all the seeds above 50 mg. and below 40 mg. were discarded. The seeds were laid out to germinate on gauze, stretched over hollow glass rings floating in water, a method used in the Botanical Laboratory of Utrecht (Holland) and giving very uniform germination. After the roots and coleoptiles had developed for a few days, a third selection was carried out. In each of the 50 two-gallon crocks six seedlings were planted; the crocks were placed in two rows of 25 along the length of the table.

The plants were supported by protected wires (pipe-cleaners) fastened to a stick placed in the center of the sand. Each plant formed 5 leaves. The tillers began to develop very late in the life cycle but were suppressed as soon as they became visible. In this way standard plants were obtained with 1 stem and 5 leaves.[1]

For every harvest an average crock was selected. In order to study the phenomena of growth in greater detail the fresh weight and dry weight of the stems + leaves and of the ears were determined separately. In the second experiment the fresh weight and dry weight of the leaves were determined as well; they were cut off along the pulvinus above the ligula. The fresh and dry weights were determined by weighing in bottles to 0.1 mg. (a) immediately after cutting and (b) after drying to constant weight at 105° C. in an oven. The outline of every leaf was traced on paper and the leaf area determined in the first experiment by weighing, in the second with a planimeter. The remaining part of the stem and sheaths is designated hereafter as "stems alone." The length of the different organs of a number of plants was measured daily in order to get an idea of the daily growth in length. Transpiration determinations with sand and water cultures were also carried out.

The number of plants harvested was 5[1] or 6, in all but two of the crocks (11 and 11R 1926) in which it was only 2. The probable errors were calculated as $r = \pm 0.6745\sqrt{\frac{\sum(\bar{x}-x)^2}{n(n-1)}}$. Though the number of individuals is too small for the calculation of significant probable errors, their values give a fair idea of the range of variability of the data.

General growth features in the two experiments with sand cultures

The first experiment was carried out in January, February and March, 1925; the second one in September, October and November, 1926.

AVERAGE DAILY TEMPERATURE

	Exp. 1925	Exp. 1926
January	22.1 ± 0.16	26.3 ± 0.08
February	22.9 ± 0.10	

AVERAGE DAILY HUMIDITY OF THE AIR

January	57.5 ± 0.88	58.1 ± 0.38
February	67.6 ± 0.75	

[1] A few plants formed 6 or 4 leaves. These plants were discarded. All the other plants were used for the determination of the mean values, except a few plants of which the total leaf area could not be determined.

The following table gives the approximate day on which the successive organs become visible:

TABLE I

GROWTH OF WHEAT IN SAND CULTURES

PLANT ORGAN	EXPERIMENT 1925 AGE OF PLANT	EXPERIMENT 1926 AGE OF PLANT
	Days	Days
1st leaves	7	5
2d leaves	9–10	7–8
3rd leaves	15	12
4th leaves	19	16
5th leaves	22	19
Ears	32	27–28
Stamens	38–40	± 30–31

In the second experiment, the temperature being 4° C. higher[2] than in the first experiment, the appearance of the successive leaves was about 3 days earlier, the appearance of the ears 4–5 days earlier, that of the stamens about 8–9 days earlier. As the plants in the second experiment reached their final size in 30 against 39 days, they were accordingly shorter (60 against 79 cm.). Every 3.5 days a new leaf appeared and in both experiments the 5th leaves appeared 14 days after the 1st leaves.

The average number of spikelets per ear in the first experiment was about 11, the number of kernels 22; in the second experiment 9 and 12, respectively. The low average number of kernels in the second experiment is probably due to the high temperature, which made several spikelets sterile; the average rate of growth, however, did not lag behind that in the first experiment. In the first experiment the kernels were ripe after 72 days, *i.e.* they showed a per cent. dry weight of 89, which means that they were air dry. The final air dry weight of the kernels developed in this experiment was about 50 mg. for the 1st and 2d spikelets and 40 mg. for the 3rd spikelets, which is a fair weight compared with the original seed (about 45 mg.). Wheat plants of the same variety sown in spring in the Experiment Garden of Stanford University were ripe after 90–100 days and had about 11 spikelets and about 30 seeds.

The first leaf persisted long after flowering. The leaves were somewhat darker green than the leaves of plants grown during spring in the field, the lower leaves were markedly longer.

The first tillers appeared only after one month, *i.e.* the stems began to develop very early; in the second experiment the first node had developed

[2] During the second experiment the temperature in the corridor, *i.e.* of the incoming air, was about 4° C. higher than during the first experiment.

on the 17th day. The third leaf is inserted at this node, the fifth and upper leaf at the third node.

The starch grains of the kernels, developed in these experiments, did not show lamellation at room temperature or after heating in water to 50° C. or higher, as has been shown by the writer (**1**). The ears of plants grown under the constant conditions did not show the typical brown color of Hard Federation, but were quite white. Plants grown from this seed in the field did not show a difference from plants grown from the original seed.

I wish to express my hearty thanks to the Directors of the Food Research Institute for the opportunity they gave me to carry out these experiments and to Dr. G. J. Peirce, who kindly provided me with working facilities in the Botany Building.

Food Research Institute and Department of Biology,
Stanford University.

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STUDIES UPON WHEAT GROWN UNDER CONSTANT CONDITIONS—II

H. L. VAN DE SANDE-BAKHUYZEN

(WITH FIVE FIGURES)

The water content of different organs throughout the life cycle

Usually the water content of an organ is given as a percentage of the dry weight or of the fresh weight. In this paper per cent. dry weight is defined as $\frac{\text{dry weight}}{\text{fresh weight}}$, and per cent. moisture as $\frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}}$ the sum of both is 100 per cent. If the increase in moisture (fresh weight minus dry weight) goes parallel with the increase in dry weight, both quotients remain the same; if the dry weight increases more rapidly than the moisture, or if water is lost, the per cent. dry weight increases, and the per cent. moisture decreases.

The stem and leaves

As fig. 1 shows, in both experiments (1925 and 1926) the percentage dry weight of the stems and leaves (ears excluded) goes up rather smoothly until the time of flowering. In experiment 1925 the plants harvested on the 41st day were the first that had flowered, flowering having occurred between the 38th and 40th days. In experiment 1926, five of the six plants harvested on the 31st day (crock 43) had not flowered and showed a dry weight of 19.5 per cent.; one plant had flowered and showed a value of 24.2. All the plants harvested after the 39th day in experiment 1925 and after the 31st day in 1926 had flowered.

The curves show evidently that there is a sudden increase in the percentage of dry weight of the stems and leaves after flowering. In 1925 this sudden increase was observed 1–2 days after flowering, in 1926 about 2–3 days after flowering (33rd day) or one day for the single plant which flowered on the 31st day.

The phenomenon that the dry weight per cent. increases or that the moisture per cent. decreases at the time of flowering does not, however, show whether this is due to a sudden increase in dry weight alone, or to an increase both in dry weight and moisture, the latter increasing less rapidly than the former, or to a sudden loss of water whether or not accompanied by an increase or decrease in dry weight. The only way to find the real cause of this change in moisture per cent. is to consider the absolute dry weight and moisture. We find then that the moisture of the stems and leaves increases until the time of flowering but decreases markedly just after flowering.

In order to make the plants harvested on successive days more comparable, an effort was made to eliminate sampling errors. In an ideal experiment no such sampling errors would have occurred, *i.e.*, all the first, second, etc., leaves would have had the same leaf area, so that the total leaf area on the same day would be the same for all plants. If we assume that after the leaves were full-grown the chemical efficiency (**2**) (assimilatory

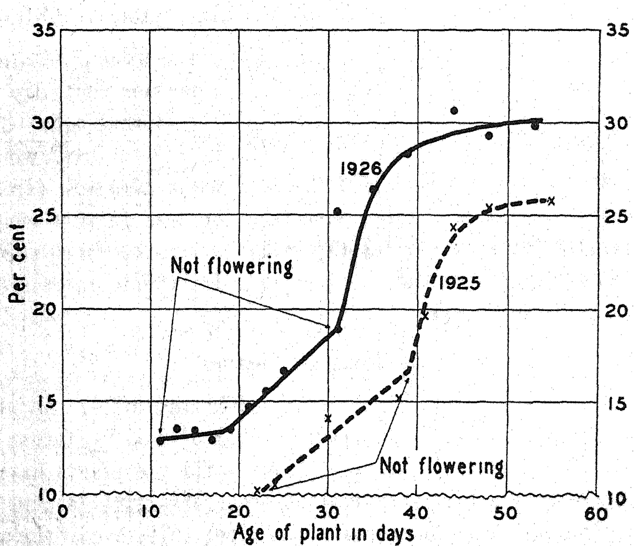

FIG. 1. Percentage dry weight of stems and leaves in the experiments of 1925 and 1926. On the 31st day of the latter experiment one of the six plants had flowered.

capacity) of the leaves and also the structural efficiency (**2**) (leaf area/total dry weight) were the same in all the plants, they would all reach the same final length. In this case we could compare the moisture of the plants in all the crocks on successive days without further calculations. In our case, however, the height and the leaf area were not the same in all plants, so that we must compare the plants harvested with an average plant. Without going into further details it is evident that the dry weight at any time depends upon the leaf area and that the correlation between both values must be 100 per cent. if both efficiencies are the same in all plants.[1] Therefore, we can to a large extent eliminate the sampling errors for the date on which all the leaves are full grown by dividing the moisture and dry weight by the leaf area.[2] The probable error of the mean values calculated

[1] In this case the leaf area and the dry weight on any day depend only upon the weight of the original seed.

[2] That we improve our data by dividing the moisture or dry weight by the total leaf area or stem length is shown by the fact that the variation coefficient of the data calculated in this way is much less than that of the original data.

TABLE II

Moisture and dry weight in mg. of stems and leaves before and after flowering. Experiment 1925

Crock no.	Age	Stem length	Moisture / Length	Dry weight / Length	Leaf area	Moisture / Leaf area	Dry weight / Leaf area
	Days	cm.			cm².		
45	38	55.2	60.7 ± 2.06	10.9 ± 0.30	62.2	53.8 ± 2.64	8.9 ± 0.50
Flowering 43	41	77.8	43.1 ± 1.01	10.7 ± 0.13	—	—	—
07	44	77.1	37.2 ± 0.83	12.0 ± 0.28	66.6	42.0 ± 1.35	13.6 ± 0.72
23	48	78.4	41.2 ± 1.51	13.8 ± 0.48	74.1	43.6 ± 2.02	14.7 ± 0.68
33	55	80.6	38.7 ± 0.58	13.5 ± 0.33	74.1	42.0 ± 0.98	14.6 ± 0.42

from the separate data of the plants in one crock may give us an idea whether this correlation is far off from 100 per cent. or not. The moisture of such a plant with unit leaf area increases until the time of flowering and then falls off suddenly, as we shall see in tables II and III. It is, however,

TABLE III

Moisture and dry weight in mg. of stems and leaves before and after flowering. Experiment 1926

Crock No.	Age	Stem length	Moisture / Length	Dry weight / Length	Leaf area	Moisture / Leaf area	Dry weight / Leaf area
	Days	cm.			cm.[2]		
14	23	37.2	48.9±2.08	9.0±0.41	56.0	32.4±0.58	6.0±0.42
39	25	42.3	—	9.2	55.9	—	6.9±0.16
43	31	59.6	45.5±1.51	10.6±0.36	65.3	41.6±0.47	9.7±0.24
Flowering	31	(61.1)	(32.4)	(11.0)	(51.7)	(38.3)	(12.9)
11	33	58.3	40.0±0.61	11.9±0.07	—	—	—
09	35	64.5	32.7±0.53	11.7±0.15	58.5	36.0±0.36	12.9±0.42
11R	39	60.7	31.7±1.02	12.6±0.05	55.8	34.5±0.71	13.7±0.24
30	44	61.1	29.4±0.68	12.9±0.38	53.9	33.4±0.60	14.7±0.32
10	48	59.2[a]	31.9±0.87[a]	12.5±0.76[a]	60.0[b]	33.6±0.51[b]	14.3±0.24[b]
41	53	60.1	31.3±1.39	13.2±0.74	55.7	33.6±0.67	14.7±0.40

[a] Average of 4 plants.

[b] Average of 2 plants, as the total leaf area of 2 plants could not be determined.

impossible to find in this way the moisture maximum, which occurs just before flowering, as the plants harvested on the 38th day (experiment 1925) and the five non-flowering plants on the 31st day (experiment 1926) were not to flower till 1–2 days later. This is shown by a comparison of stem length (ear excluded) and leaf area. In 1926 for all the plants harvested after flowering (21 plants) the ratio stem length/leaf area is 1.06 ± 0.015, while for the five non-flowering plants in crock 43 it is 0.92 ± 0.03, proof that the stems in the latter crocks are not yet full grown. We come to the same result if we compare the lengths of the successive internodes. As is generally known the length of the n^{th} node is about the arithmetic mean of the $(n-1)^{st}$ and $(n+1)^{st}$ internode. If we calculate the ratio of the length of the (2d + 4th [ear excluded]) internode and twice the length of the 3rd internode, we find in 1926 for the flowering plants an average of 1.08, while for the five non-flowering plants on the 31st day it is 1.00. In 1925 the latter values for the flowering plants are 1.09, for crock 45 (38th day), which has not yet flowered, 0.69. Therefore, if there is a loss

of water at the time of flowering, it will be larger than is found by dividing the moisture by the leaf area, as the maximum moisture found is less than the real maximum moisture.

If we divide the moisture by the stem length, we assume that the massive cylinder of tissue in the stem around the central cavity is of uniform radius and thickness in all plants and in all internodes; the last internode, however, is much thinner than the lower ones. Therefore in full-grown plants the average moisture and dry weight per cm. stem length must be smaller than in plants in which the last internode is not yet full grown. Moreover, the moisture per cent. decreases in the successive internodes. Thus we find too high a value for the loss of water.

We may expect, therefore, that the true loss of water lies between the value for the loss found by dividing the moisture by the leaf area and the value found by dividing it by the stem length. In tables II and III both sets of values are given. In crock 14 (experiment 1926), harvested on the 23rd day, all the leaves were full grown, but the ears were not yet formed, so that the moisture and the dry weight of the entire tops were divided by the leaf area or the length of the plant from the base up to the 5th (last) ligula. The latter value has, therefore, only a relative significance, as it is not quite comparable with the values of the plants in which the stems were nearly or quite full grown.

Tables II and III show that in both experiments 1925 and 1926 there occurs a sudden loss of water within 1–2 days after the time of flowering. In experiment 1925 the moisture of stems + leaves calculated per cm. stem length dropped after flowering from 60.7 $\pm$ 2.06 mg. (38th day) to 43.1 $\pm$ 1.01 mg. (41st day), which means a loss of 29 per cent. of the moisture present on the 38th day. The moisture calculated per cm^2. leaf area drops from 53.8 $\pm$ 2.64 mg. (38th day) to 42.0 $\pm$ 1.35 (41st day), which equals a loss of 22 per cent. The true loss lies between 22 and 29 per cent. After the 41st day, *i.e.* 1–2 days after flowering, the values of the moisture decrease slightly or remain practically constant until the 55th day.

The same result was obtained in experiment 1926, as is shown in table III. In the five non-flowering plants of crock 43 (31st day) the moisture per cm. stem length is 45.5 $\pm$ 1.51 mg., which is in fair agreement with the value of crock 14 (23rd day) : 48.9 $\pm$ 2.08 mg. The one flowering plant in crock 43 (the values for this plant are given in parentheses) shows a moisture per cm. stem length of 32.4 mg. which agrees closely with the value of crock 09 (35th day). The values of crock 11 (2 plants only) shows an intermediate position between crocks 43 and 09. The value of crock 09 shows a loss of 27 per cent. compared with the moisture before flowering. After the 35th day the moisture decreases slightly until the 53rd day. The moisture calculated per $cm.^2$ leaf area increases from the 23rd day

(32.4 ± 0.58 mg.) until the time of flowering (41.6 ± 0.47 mg.) and decreases 1–5 days after flowering (38.3 mg. for the one flowering plant in crock 43 and 36.0 ± 0.36 mg. in crock 09). The loss of water in crock 09, calculated in this way, is 13.5 per cent. of the moisture present in the five plants of crock 43. There is still a further decrease between the 35th and 44th day and then the value remains constant until the 53rd day. The true loss of water between the 31st day and the 35th day lies between 13.5 and 27 per cent. The dry weight of stems and leaves increases all the time. In experiment 1926 the mean value of the dry weight on the 33rd day was probably found too high, moreover the plants (only 2) were very short. Therefore the moisture on this day also may be too high (40.0 ± 0.61), which is the more probable if we compare this value with the values of the single flowering plant in crock 43 and of the plants in crock 09.

In experiment 1926 the probable errors of the moisture and dry weight per unit stem length are 3 per cent. of the total value; if calculated per unit leaf area they are 2.2 per cent. In experiment 1925 these values are respectively 2.6 and 4.2 per cent. The latter value in 1925 (4.2) is higher than in 1926 (2.2), because in 1925 the leaf area was determined by weighing paper tracings, which gave less accurate results than the determination with a planimeter in 1926. It seems, therefore, that the best results are obtained by dividing the total moisture or dry weight of stems + leaves by the leaf area. This corresponds with the fact that the probable errors of the values per unit leaf area for the "leaves alone" are very low, as will be evident later.

The "stems alone"

For the values of the percentage of dry weight of the stems alone (stems + sheaths) the same holds as for the stems + leaves; this is shown in fig. 2, and in table IV. The single flowering plant in crock 43 had a much higher dry weight per cent. (27.5) than the five non-flowering plants (18.9). Crock 09, harvested 4 days later, shows a value of 28.1. It is evident that there is a sudden rise in the values just after flowering.

Whether in the stems alone the absolute weights of moisture and dry weight, or the weights per unit stem length or unit leaf area be employed, exactly the same results are obtained as when these calculations are made for the stems + leaves. The values for the moisture per cm. stem length on the 23rd and the 27th days agree fairly well with the value of the five non-flowering plants on the 31st day, being respectively 30.4 ±1.11, 29.3 ± 2.11 and 31.7 ± 1.12 mg. The values on the 27th day are too low, and indicate not merely for the stems but also for the leaves an abnormally low moisture and dry weight. This is probably due to the fact that this crock had accidentally a soil moisture which was much too low; the values for the plants

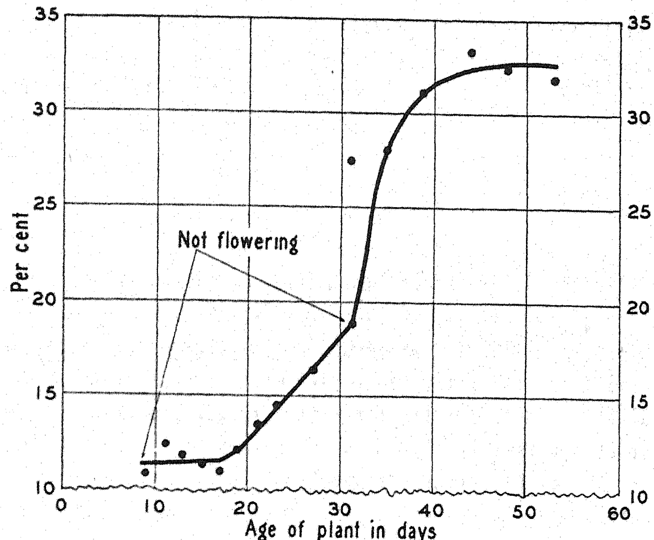

FIG. 2. Percentage dry weight of stems alone. Experiment of 1926. On the 31st day one of the six plants had flowered.

were not very uniform and this is reflected in a very high probable error. The one flowering plant on the 31st day shows a value (22.7 mg.) which agrees closely with the value on the 35th day (22.4 ± 0.38 mg.). After the 39th day the moisture and the dry weight remain constant until the 53rd day.

TABLE IV

MOISTURE AND DRY WEIGHT IN MG. OF STEMS ALONE BEFORE AND AFTER FLOWERING. EXPERIMENT 1926

CROCK NO.	AGE	STEM LENGTH	MOISTURE / LENGTH	DRY WEIGHT / LENGTH	LEAF AREA	MOISTURE / LEAF AREA	DRY WEIGHT / LEAF AREA
	Days	cm.			cm.^{2}		
14	23	37.2	30.4±1.11	5.1±0.21	56.0	20.2±0.54	3.4±0.11
39	25	42.3	—	5.7	55.9	—	4.1±0.11
42	27	42.5	29.3±2.11	5.7±0.33	—	—	—
43	31	59.6	31.7±1.12	7.4±0.29	65.3	29.0±0.40	6.8±0.24
Flowering		(61.1)	(22.7)	(8.6)	(51.7)	(26.8)	(10.1)
09	35	64.5	22.4±0.38	9.0±0.20	58.5	24.7±0.35	9.8±0.32
11R	39	60.7	21.5±0.78	9.8±0.07	55.8	23.4±0.54	10.7±0.24
30	44	61.1	20.1±0.38	10.1±0.33	53.9	23.3±0.67	11.5±0.30
10	48	59.2[a]	21.5±0.61[a]	10.0±0.43[a]	60.0[b]	22.7±0.59[b]	11.1±0.24[b]
41	53	60.1	21.7±0.98	10.1±0.54	55.7	23.4±0.49	10.9±0.28

[a] Average of 4 plants.
[b] Average of 2 plants; see table III.

The same holds for the moisture and dry weight calculated per unit leaf area. For the reasons above given, the loss of water calculated in this way is smaller than when calculated per unit stem length, the loss on the 35th day (crock 09) being respectively 15 per cent. and 30 per cent. of the moisture present in the five non-flowering plants on the 31st day. The true loss up to the 35th day lies between 15 and 30 per cent.

The dry weight per unit leaf area increases more than does the dry weight per unit stem length as the leaf area remains constant after the 23rd day, while the dry weight of the stem increases proportionately more with its length, though the dry weight per unit length also increases.

If in the "stems alone" the values of fresh weight and dry weight are calculated per unit leaf area, the probable errors are absolutely and relatively smaller (respectively 2.1 per cent. and 2.7 per cent.) than if calculated per unit stem length (respectively 3.5 per cent. and 3.4 per cent.), as was also the case for the stems + leaves.

For the plants in experiment 1925 no data are available for the stems alone.

The ears

There is some evidence that in the ears there is also a sudden loss of water at the time of flowering. This is indicated in both experiments not only by a rapid increase in the percentage of dry weight between 1–2 days before flowering and 2 days after flowering, but we find this loss also, if we consider the absolute values of moisture and dry weight.

The same results are obtained if the moisture per unit ear length or per unit leaf area be calculated. In the experiment of 1925 the moisture increases again 4–5 days after flowering; in that of 1926 it increases 5 days after flowering, so that on the 39th day the original moisture is exceeded again. The data are given in table V.

TABLE V

MOISTURE AND DRY WEIGHT OF EARS IN GM. BEFORE AND AFTER FLOWERING

Crock no.	Experiment 1925				Experiment 1926				
	Age	Moisture	Dry weight	No. of spikelets	Crock no.	Age	Moisture	Dry weight	No. of spikelets
	Days	gm.	gm.			Days	gm.	gm.	
Not flowering ... 45	38	0.519	0.130	10.5	43	31	0.5910	0.1722	10.4
Flowering 43	41	0.431	0.193	10.6	43 II[a]	31	0.4508	0.1495	9.0
07	44	0.511	0.267	11.0	11	33	0.4852	0.1738	10.5
					09	35	0.4961	0.2207	9.3
					11 R	39	0.5967	0.2860	9.0

[a] 43 II means plant II of crock no. 43.

The leaves

The percentage of moisture of the leaves decreases as we ascend from the lower to the upper leaves, *i.e.*, the latter have relatively more dry weight and less moisture than the former. Table VI shows the differences in dry weight per cent. in the successive leaves harvested on different days (experiment 1926).

TABLE VI

PERCENTAGE DRY WEIGHT OF LEAVES EXPERIMENT 1926

AGE	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES
Days	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
23	15.1	14.6	16.0	18.0	20.2
35	18.0	17.1	19.3	22.0	24.7
53	24.4	21.2	21.7	24.4	27.0

This table shows that though the first two leaves show about the same per cent. dry weight, this value increases from the 2d leaf to the 5th. On the 53rd day the value for the 1st leaf is much higher than that of the 2d, because the former is yellowing and gradually dying off. For the other days and in experiment 1925 this difference in percentage of dry weight in the successive leaves is just as striking. Table VII gives these values for the upper and lower leaves of tomatoes (table XXIV, KRAUS and KRAYBILL (7)) and of leaves of corn (SCHWEITZER (**11**)).

TABLE VII

PERCENTAGE DRY WEIGHT OF LEAVES OF TOMATO AND CORN QUOTED FROM KRAUS AND KRAYBILL AND SCHWEITZER

PLANT	LOWER LEAVES	UPPER LEAVES
	Per cent.	Per cent.
Tomatoes	14.00	18.60
Corn (68 days old)	12.30	14.13

Fig. 3 shows the percentage dry weight of the 3rd leaves harvested on successive days (experiment 1926). The value increases approximately rectilinearly until the time of flowering. The one flowering plant in crock 43 shows a much higher value than the five non-flowering plants.

It is evident that the value on the 35th day is much higher than could be expected from the trend before flowering. After the 35th day the trend is approximately the same as before flowering, only at a higher level. The same holds for the other leaves. Again the dry weight and moisture per $cm.^2$ of leaf area must be calculated in order to eliminate sampling errors. Usually only the percentage of moisture or percentage of dry weight is given in the literature. JOST (3) quotes a case where erroneous results are obtained if the morning and evening N-content of leaves is calculated per unit leaf dry weight, because a unit leaf area weighs less in the morning than it did the previous evening. Moreover, in calculating the apparent

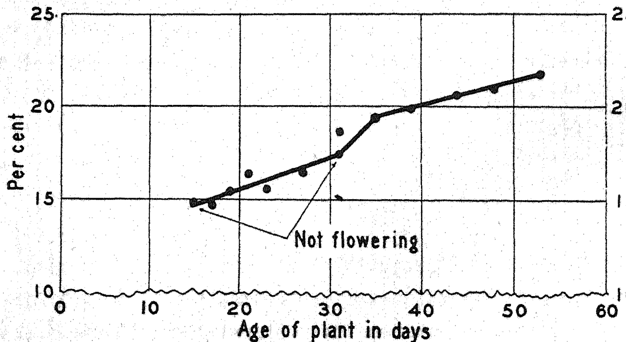

FIG. 3. Percentage dry weight of the 3rd leaves. Experiment of 1926. On the 31st day one of the six plants flowered.

assimilation of an entire plant it is usually advantageous to calculate the daily increase in dry weight per unit leaf area instead of per unit leaf dry weight. Though we are quite aware that it is much more laborious to calculate the moisture by the former method than by the latter, we think this latter method should be employed to a much greater extent than it has been heretofore.

In evaluating the moisture or dry weight per $cm.^2$ of leaf area, the order of magnitude of the probable error to be expected must first be considered. In our second experiment the fresh weight and dry weight were determined with an error of about 0.1 mg., *i.e.*, the probable error of the moisture is about 0.1–0.2 mg. The outlines of the leaves were traced on paper. What errors were made in tracing was not determined, though it is probable that in the tracing of the lower leaves a relatively larger error was made than in the tracing of the upper leaves. In the determination of the leaf area with a planimeter an average error of $\pm$ 0.1 $cm.^2$ was made; every leaf was measured three times and if the values differed more than $\pm$0.1 $cm.^2$, more measurements were made. An error of $\pm$ 0.1 $cm.^2$ leaf area affects the dry weight per $cm.^2$ in the second decimal of a milligram and the moisture to an extent of $\pm$ 0.1 mg.

If the total dry weight of every leaf be divided by its leaf area, the assumption is made that every leaf has the same thickness and structure, *i.e.,* that there exists a correlation of 100 per cent. between leaf area and dry weight or moisture. Though this correlation has not been calculated, it is reflected in the low values of the probable errors, which are about 1.5 per cent. of the mean values per $cm.^2$, which is much less than found in the stems and leaves or stems alone.[3] In a few cases, however, in which the leaf areas are very large or very small there is a slight correlation between the leaf area and the values per $cm.^2$, proof that these leaves are different in thickness or structure.

The average dry weight per $cm.^2$ of leaf area of all the leaves is 2.5 ± 0.04 mg., the average moisture 11 to 12 ± 0.18 mg. (tables VIII and IX). The largest deviation from the mean moisture very rarely exceeds 0.8–1.0 mg. (except for the single flowering plant of crock 43). In plants harvested on the same day the values of dry weight and moisture can be compared with checks and counter checks as the following relations are known.

1. Leaf area and leaf dry weight (dry weight per $cm.^2$ leaf area).
2. Leaf dry weight and fresh weight (percentage of dry weight or percentage of moisture).
3. Leaf area and leaf moisture (moisture per $cm.^2$ leaf area).
4. Dry weight of a single leaf and dry weight of adjacent leaves, or dry weight of the entire plant.

The correlation between the dry weight of the 1st leaf and the dry weight of stems or entire tops exceeded in many cases 0.90.

If 1 shows a considerable deviation from the mean (13 per cent. of the 237 cases showed a deviation larger than 0.2 mg.), this is an indication that probably a mistake has been made in tracing the leaf area, as in these cases the moisture per $cm.^2$ is also abnormal; the percentage of dry weight may be quite normal. An abnormality in water content is reflected in moisture per $cm.^2$ and percentage of moisture.

Aside from these abnormal values, the values are very consistent, as shown not merely by the low probable errors, but also by the mean values for the crocks harvested on successive days, as shown in tables VIII and IX. All the leaves harvested[4] were used for the determination of the mean values and the probable errors. The value for the dry weight of the 5th leaves of crock 41 is certainly too low, as one plant showed a value of 3.1; if we

[3] See also footnote 2, p. 8.

[4] A few 5th leaves were too much curled to allow accurate determination of the leaf area.

omit this value the dry weight would be 4.0 ± 0.14 mg., which is more in agreement with the values on the previous days.

All the leaves of the plants harvested were full grown except the 4th leaves of no. 46 and the 5th leaves of no. 23, which are placed between brackets.

TABLE VIII

LEAF MOISTURE IN MG. PER CM.[2] LEAF AREA. EXPERIMENT 1926

CROCK NO.	AGE	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES
	Days	mg.	mg.	mg.	mg.	mg.
45	9	13.1 ± 0.35				
26	11	12.6 ± 0.30				
21	13	12.8 ± 0.16	12.2 ± 0.18			
12	15	12.8 ± 0.17	12.3 ± 0.18	11.7 ± 0.11		
37	17	12.9 ± 0.70	12.7 ± 0.20	11.7 ± 0.17		
46	19	14.1 ± 0.39	12.8 ± 0.27	12.1 ± 0.22	[12.1 ± 0.13]	
23	21	14.7 ± 0.34	12.9 ± 0.21	12.2 ± 0.11	11.6 ± 0.15	[12.1 ± 0.46]
14	23	13.8 ± 0.07	13.1 ± 0.28	12.3 ± 0.13	11.8 ± 0.17	11.7 ± 0.28
39	25	—	—	—	—	—
43	31	14.1 ± 0.11	12.9 ± 0.06	12.9 ± 0.23	12.6 ± 0.13	12.0 ± 0.16
		(12.1)	(12.3)	(11.4)	(11.2)	(11.0)
09	35	12.1 ± 0.11	12.5 ± 0.15	11.6 ± 0.19	10.9 ± 0.13	10.4 ± 0.08
11R	39	—	11.4 ± 0.20	11.2 ± 0.37	10.7 ± 0.13	10.3 ± 0.11
30	44	9.6 ± 0.35	11.2 ± 0.08	10.9 ± 0.17	10.7 ± 0.10	9.9 ± 0.17
10	48	10. 9± 0.20	10.9 ± 0.11	10.9 ± 0.14	10.5 ± 0.18	10.4 ± 0.05
41	53	8.5 ± 0.20	10.8 ± 0.12	10.7 ± 0.13	10.6 ± 0.19	10.4 ± 0.07
Average probable error		± 0.21	± 0.17	± 0.18	± 0.15	± 0.13

In crock 43 ( ) means one plant flowering, the five other plants not yet flowering. After the 32–33rd day all the plants have flowered.

[ ] means leaves not yet full grown.

If the tables are read left to right, we find that the dry weight per cm.[2] increases while the moisture per cm.[2] decreases. If this be expressed in terms of percentage of dry weight, the values increase from the lower leaves to the upper leaves (table VI). Before flowering, the moisture of second leaves is always lower than the moisture of first leaves, but the dry weight of the former is often equal to or lower than that of the latter. Thus the second leaf often has a lower per cent. dry weight than the first leaf, as found above. The average variation coefficients for the dry weights of the successive leaves are approximately the same; for moisture, the coefficients decrease markedly, because the absolute probable error (or standard deviation) decreases more than the mean values themselves decrease as we ascend from the first to the fifth leaf.

TABLE IX

LEAF DRY WEIGHT IN MG. PER CM.² LEAF AREA. EXPERIMENT 1926

CROCK NO.	AGE	1ST LEAVES	2D LEAVES	3D LEAVES	4TH LEAVES	5TH LEAVES
	Days	mg.	mg.	mg.	mg.	mg.
45	9	1.9 ± 0.03				
26	11	2.0 ± 0.02				
21	13	2.2 ± 0.05	2.2 ± 0.03			
12	15	2.1 ± 0.02	2.2 ± 0.04	2.05 ± 0.02		
37	17	2.1 ± 0.10	2.2 ± 0.03	2.0 ± 0.03		
46	19	2.2 ± 0.06	2.2 ± 0.02	2.2 ± 0.02	[2.3 ± 0.04]	
23	21	2.4 ± 0.02	2.4 ± 0.03	2.3 ± 0.05	2.5 ± 0.04	[2.9 ± 0.05]
14	23	2.3 ± 0.05	2.4 ± 0.02	2.4 ± 0.01	2.6 ± 0.05	3.0 ± 0.07
39	25	2.4 ± 0.01	2.4 ± 0.01	2.6 ± 0.02	2.7 ± 0.05	3.0 ± 0.02
43	31	2.8 ± 0.09	2.5 ± 0.02	2.7 ± 0.02	2.8 ± 0.05	3.4 ± 0.04
		(2.6)	(2.5)	(2.6)	(2.8)	(3.2)
09	35	2.7 ± 0.06	2.6 ± 0.04	2.8 ± 0.01	3.1 ± 0.01	3.5 ± 0.09
11R	39	2.7 ± 0.0	2.7 ± 0.0	2.8 ± 0.05	3.1 ± 0.0	3.5 ± 0.06
30	44	2.7 ± 0.04	2.7 ± 0.02	2.8 ± 0.02	3.4 ± 0.06	4.0 ± 0.08
10	48	2.9 ± 0.04	2.7 ± 0.07	2.9 ± 0.05	3.2 ± 0.07	3.9 ± 0.09
41	53	3.0 ± 0.10	2.9 ± 0.10	3.0 ± 0.06	3.4 ± 0.04	3.8 ± 0.16
Average probable error		± 0.05	± 0.03	± 0.03	± 0.04	± 0.08

In crock 43 ( ) means one plant flowering, the five other plants not yet flowering. After the 32–33rd day all the plants have flowered.
[ ] means leaves not yet full grown.

Reading the table of the dry weights from above downward, we find that the values for all the leaves increase throughout the life cycle.

If we read the table of the moisture from above downward, we see that this value increases for all the leaves up to the time of flowering, the five non-flowering plants in crock 43 showing the highest figures. But the relative increase in moisture is less than the relative increase in dry weight, so that the dry weight per cent. increases from the beginning until the time of flowering, as shown in fig. 3.

The single flowering plant (43 II) in crock 43 (between brackets) shows a considerably lower moisture. That this is not an accidental deviation is shown by the fact that the stems and the ears also had a lower moisture content. This is also shown by the values of crock no. 09, harvested four days later, as shown in table X.

In order to ascertain whether the difference between 43 II and the mean of the five non-flowering plants and also the difference between the five non-flowering plants and the one flowering plant in no. 43 + the six plants in crock 09 (which had flowered) is significant, how many times this differ-

TABLE X

LEAF MOISTURE IN MG. PER CM.[2] LEAF AREA OF PLANTS WHICH HAD PRODUCED STAMENS

CROCK NO.	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES
43 II	12.1	12.3	11.4	11.2	11.0
09	12.1	12.5	11.6	10.9	10.4

ence exceeds the probable error can be calculated. As the number of samples is, however, small, it is preferable to use the method of FISHER (**6**), which determines the probability (1–P) that a difference between both values, larger than the difference observed, will occur.[5]

$$t = \frac{(\bar{x}_1 - \bar{x}_2)\sqrt{(n_1 + n_2 - 2)}}{\sqrt{(S_1 + S_2)}} \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

In a table the value P corresponding to different values of t can be found. In this formula

$\bar{x}_1$ = mean of one set (n_1) of observations (*i.e.*, non-flowering plants),

$\bar{x}_2$ = mean of another set (n_2) of observations (*i.e.*, flowering plants),

S_1 = sum total of the square of the deviations from $\bar{x}_1$,

S_2 = sum total of the square of the deviations from $\bar{x}_2$.

In table XI the values (1–P) are given for the differences observed between the flowering and non-flowering plants.

TABLE XI

PROBABILITY (1–P) THAT A DIFFERENCE EQUAL OR LARGER THAN THE DIFFERENCE OBSERVED BETWEEN FLOWERING AND NON-FLOWERING PLANTS WILL OCCUR

PLANTS COMPARED	1ST LEAVES	2D LEAVES	3RD LEAVES	4TH LEAVES	5TH LEAVES	STEMS ALONE
43 non-flowering and 43 flowering ...	0.03	0.03	0.07	0.02	0.075	0.045
43 non-flowering and 43 II + 09	<0.0003	0.077	0.0084	0.0001	<0.0002	0.0002

[5] This method was kindly suggested to me by Dr. H. HOTELLING, of the Food Research Institute.

If the value (1–P) = 0.05 the difference is suggestive
= 0.02 the difference is significant
= 0.01 the difference is very significant

Table XI shows that in crock 43 a significant difference exists between the 1st, 2d, and 4th leaves, and probably the stems of flowering and non-flowering plants, and that between the five non-flowering plants of 43 and the six plants in 09 + the flowering plant of 43 (43 II) a very significant difference exists except for the 2d leaves. That the difference in the 3rd leaves, 5th leaves and stems are not significant is due to plant 43 I, which shows rather low moisture values, though not so low as the flowering plant 43 II. This plant had not yet produced stamens, but the ratio stem length/leaf area was 1.07, which is equal to the mean of the plants which had staminated (1.06 ± 0.015) while the four other non-flowering plants in this crock showed a ratio of 0.88 ± 0.02. This proves that this plant was near to the flowering stage. If this one plant be omitted, then the values for (1–P) become much more significant. It is difficult to say whether in this plant the low value of the moisture was due to beginning of water loss or not. Moreover, our data do not show at exactly what moment the loss of water from the leaves, stems and ears occurs, whether at stamination, at fertilization of the ovaries, or earlier.

Fig. 4 and fig. 5 show graphically the dry weight and moisture per $cm.^2$ of the different leaves. Fig. 5 brings out the fact that the dry weight increases as we ascend from the 2d to the 5th leaves; the smoothed values for the 1st leaf are somewhat higher than the values for the 2d leaf. Furthermore, it can be seen that after flowering the increase in dry weight in the 4th and 5th leaves is much larger than in the 1st, 2d, and 3rd leaves.

Fig. 4 shows clearly that the moisture decreases from the 1st leaf to the 5th leaf. The 1st and 2d leaves show constant values 10 days before flowering; in the 3rd, 4th and 5th leaves the values increase continuously until flowering. The slope of the curve is less steep the higher the order of the leaf. In table XII is shown the number of days from the appearance of the leaves to the time of maximum moisture content.

This table shows that the number of days during which the moisture increases is the same for the first two leaves, perhaps also for the 3rd and 4th leaves. In the 1st leaves the moisture remains constant for 12 days (19th–31st day), in the 2d leaves for 10 days (21st–31st day). This plateau does not occur in the 3rd–5th leaves as the flowering period interferes.

After flowering the drop in moisture is most pronounced and continuous in the 1st leaves; in the other leaves the decrease slows down earlier the higher their order. In this way the moisture in the 4th leaves remains

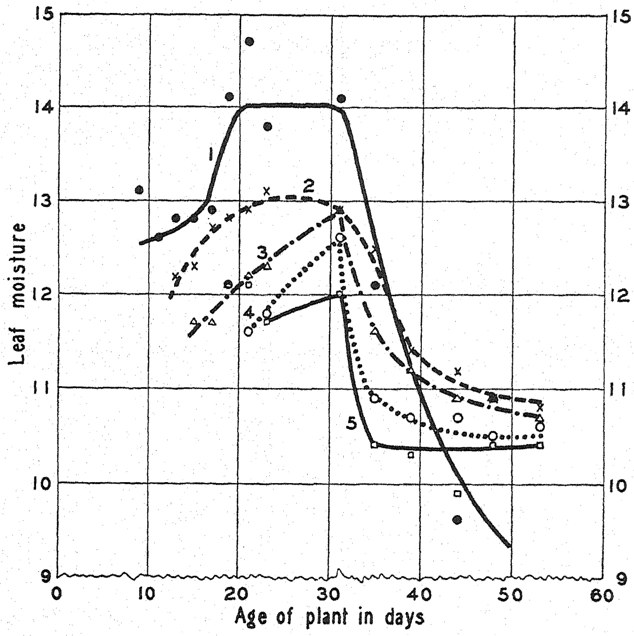

FIG. 4. Moisture of the successive leaves in mg. per cm.² leaf area. Experiment of 1926. See table VIII. The one flowering plant on the 31st day is omitted.

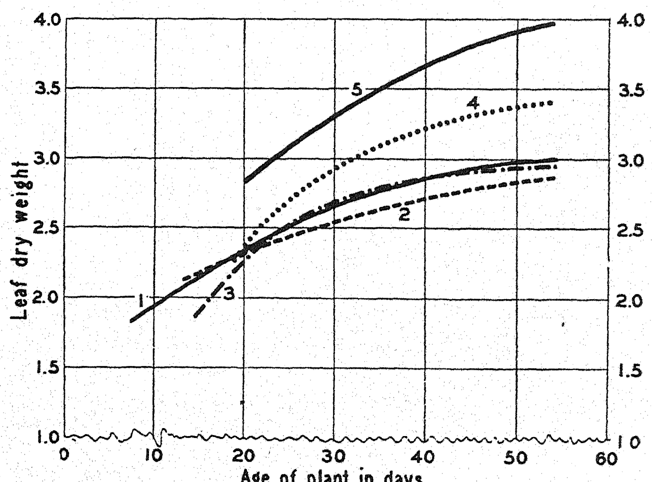

FIG. 5. Dry weight of the successive leaves in mg. per cm.² leaf area. Experiment of 1926. See table IX. The one flowering plant on the 31st day is omitted.

TABLE XII

NUMBER OF DAYS DURING WHICH THE MOISTURE IN THE SUCCESSIVE LEAVES INCREASES. EXPERIMENT 1926

LEAF NO.	DAY OF APPEARANCE	DAY ON WHICH THE HIGHEST VALUE WAS REACHED	NUMBER OF DAYS DURING WHICH MOISTURE INCREASES
1st	5th	19th	14
2d	7–8th	21st	13–14
3rd	12th	23–31st	11–19
4th	16th	23–31st	7–15
5th	19th	23–31st	4–12

about constant after the 39th day, in the 5th leaves after the 35th day. Apparently the lower leaves are more affected by the flowering than the higher leaves. It is possible that the fact that after flowering the lower leaves gain less in dry weight than the upper ones, has to be explained in this way. The external evidence of a decrease of water content is shown by the gradual yellowing and dying of the leaves. While on the 31st day 16 per cent. of the first leaves and 16 per cent. of the 2d leaves showed yellow tips, on the 35th day 66 per cent. of the 1st leaves, and 33 per cent. of the 2d leaves showed such tip. The 3rd, 4th, and 5th leaves did not exhibit yellow tips on the 35th day, though their moisture had decreased; the first yellow tips were visible on the 39th day. Therefore the yellowing of the tips cannot account for the decrease in moisture,[6] but the continuous deficit in moisture in the leaves is the principal cause of their yellowing and dying.

Determinations after the 53rd day were not made, as the lower leaves began to die rapidly and the yellowing of the upper leaves increased.

Discussion

The tables given in this paper show plainly that in the different cases a change in percentage of dry weight must be explained in different ways. The fact that the higher leaves show a higher dry weight per cent. is due to their higher dry weight and lower moisture per unit leaf area. The dry weight per cent. of every leaf increases throughout the life cycle. Before the flowering stage this is due to the fact that both dry weight and moisture increase, but the former increases more rapidly than the latter; after the flowering stage the moisture decreases while the dry weight continues to increase. Probably exactly the same holds for the "stems alone." In

[6] Even if these tips had contained no water at all, this could not account for the absolute quantity of water loss that was observed.

experiment 1925 the dry weight per cent of the successive internodes was determined on the 48th day, *i.e.*, about 9 days after flowering.

TABLE XIII

PERCENTAGE DRY WEIGHT OF SUCCESSIVE INTERNODES (48TH DAY, EXPERIMENT 1925)

1st internode + 1st sheath + 2d sheath + coleoptile	29.0 per cent.
1st node + 2d internode + 3rd sheath	22.0 per cent.
2d node + 3rd internode + 4th sheath	23.3 per cent.
3rd node + 4th internode + 5th sheath	29.5 per cent.

Though probably the first internode always shows a somewhat higher dry weight per cent. than the 2d internode (as is the case in the 1st and 2d leaves, fig. 5), the high value of the former in table XIII is certainly due to the fact that this internode loses more water after flowering than do the higher internodes (as is also the case in the leaves, fig. 5). This corresponds with the fact that the first leaves with sheaths, and the 1st internodes, had already begun to yellow at this time.

This analysis of dry weight per cent. data shows that a change in this value can be brought about very differently in different cases. It is strictly necessary to express the total absolute moisture and dry weight or to calculate the values per unit length or area. This analysis shows furthermore that it is not permissible to compare the dry weight per cent. of leaves in different plants without taking into consideration the order of the leaves.

In experiment 1926 the temperature was about 4° C. higher than in 1925; therefore transpiration must have been much higher in 1926. The dry weight per cent. in 1926 was always higher than the same value in 1925. Thus this value 1–2 days before flowering was 16.1 per cent. in experiment 1925 (38th day); 19.5 per cent. in experiment 1926 (five non-flowering plants 31st day). After flowering, these differences between the values in both experiments persisted. In 1926 the leaves and ears emerged earlier; the leaf area and stem length were less than in 1925. The rate of growth of the stems, however, seems to have been nearly the same in both experiments, as in 1925 a final length of 79 cm. was reached in 39 days, in 1926 a length of 60 cm. in 30 days. Therefore the differences between the two experiments must be ascribed in part to a difference in growing period between sowing and flowering. But parallel with a shorter growing period there goes a more rapid increase of the percentage of dry weight. We think that this higher percentage of dry weight (or lower percentage of moisture) is the cause of the shortening of the period between sowing and flowering. The dry weight per cent. is certainly an important physiological index. In our experiment it was very uniform in different plants of the

same age. In 1925 this value for the entire top was 14.0 ± 0.10 per cent. (average of nine plants) on the 30th day.

The dry weight of the leaves shows a continuous increase throughout the life cycle. Though the leaves may be full grown insofar as a constant leaf area is reached, they still increase in weight. DIXON (**4**) found that older leaves showed a higher osmotic pressure than younger leaves. URSPRUNG and BLUM (**16**) found the osmotic value (saltpeter value) in the younger leaves of *Urtica* smaller than in older ones; the value in the stem of *Urtica* and *Helleborus* decreases from the base towards the top. WILLSTÄTTER (**15**) showed that the chlorophyll content of leaves may increase for several weeks. The explanation of the different dry weight and moisture of the successive leaves must wait until further anatomical studies have been made. The marked sequence of the values for the dry weight and moisture of every leaf and their low probable errors proves that in different plants the leaves of the same order are very uniform in structure and physiological behavior. In other words, the sampling error has been practically eliminated.

While the first parts of the dry weight curves of the successive leaves are curves with increasing slope, the last parts of these curves, as represented in figs. 4 and 5, exhibit a decreasing slope (S-shaped curve).

The increase in dry weight throughout the life cycle is also found in the stems. Though the higher internodes are thinner, the average dry weight per cm. stem length increases continuously. This increase lasts for a certain time after flowering, as is also the case in the leaves.

The increase in moisture and dry weight in the leaves and stems which have completed their increase in area or length is probably due to a further increase in volume of the parenchyma cells.[7]

The moisture in the stems and in the leaves increases continuously until the flowering stage interferes. After flowering, with the increase of dry weight in these organs, there is no longer increase in moisture, but the opposite, *viz.*, a sudden loss of water. That after flowering the lower leaves gain less in dry weight than the upper ones may be explained by the fact that the lower leaves suffer more from water loss than the upper (table IX and fig. 5). Indeed, several investigators [literature cited in (**2**)] have shown that photosynthesis depends largely upon the water content of the leaves. On the other hand, it is possible that the lower leaves were physiologically full grown, while the upper leaves, developing later, are still capable of increasing in weight. Owing to a more pronounced and continuous deficit in water content, the lower leaves are the first to die.

The same probably holds for the stems in which the first internode is the first to yellow.

[7] This has been pointed out by BERTHOLD (Unt. z. Phys. der Pflanzl. Organization 2: I. 1904.

The water loss in the ears lasts only a few days as the kernels begin to swell, resulting in a marked increase in dry weight and moisture.

The increase in dry weight in the different organs will be discussed in a later paper. We may, however, anticipate so far as to state that the loss of water in the leaves and other assimilatory organs must affect photosynthesis, thus depressing the total increase in dry weight. In the field this should be the more pronounced the drier the soil and air. Moreover, in the yellowing leaves the rate of photosynthesis must fall off. This results in a decrease in the slope of the growth curve (last part of the total S-shaped curve), which is the more accentuated as the leaves gradually die from the base towards the top (**1**).

The cause of this sudden loss of water at the time of flowering or fertilization must now be considered. Experiments with sand and with water cultures indicated that the increase in transpiration, corresponding to the growth of the last internode, ceases 1–2 days before the plants produce stamens. The loss of water probably occurs 1–2 days after flowering. It is not probable, therefore, that the loss of water is due to the fact that the ears while growing came nearer to the lamps resulting in an increase in transpiration.[8] Moreover, in crock 43 the flowering plant (61.1 cm. long) was not the tallest one, two non-flowering plants were respectively 64.3 and 60.4 cm. long while their moisture was practically equal to or higher than the mean of the non-flowering plants. Moreover, several plants after flowering were shorter than 59.6 cm. (the average of the five non-flowering plants in crock 43), but showed nevertheless a pronounced loss of moisture. Furthermore, the loss of water at this time is certainly a general phenomenon in annuals grown in the field as data in the literature show (**2**). Therefore, the flowering stage must be an important moment in the life cycle of an annual or biennial and we might expect cloudy or rainy weather to be favorable at this time (**1**). On the other hand, rain may be harmful at this stage through washing nitrates, etc., out of the soil, thus complicating the answering of the question whether or not the flowering stage is a critical period in the life cycle. At any rate, the fact that the drying out of an annual begins as soon as the young sporophytes begin to develop must be considered.

The growth in length of the internodes ceases on the same day the stamens appear, *i.e.*, when pollination takes place. A similar phenomenon was studied by MURNEEK (**9**) in tomatoes. He found that vegetative growth was inhibited by developing fruits. This inhibition was more pronounced the greater the number of the fruits that were developing. There

[8] The lamps were renewed in both experiments at such a time as not to interfere with the flowering period.

is, however, a difference between the case of wheat and of the tomato. The fruits of tomatoes are lateral, in wheat apical; the inhibition of vegetative growth in tomatoes occurs while the fruits are developing, in wheat at flowering. MURNEEK ascribes this phenomenon in tomatoes to the fact that the fruits monopolize the nitrogen. As was pointed out in a previous paper (**2**), it is preferable to say that the cells in the fruits are in such a state that they can take in and utilize nitrogen whereas the cells in the vegetative parts cannot. That these parts are still able to grow is proven by the fact that this inhibition depends to a large extent upon the nitrogen given to the plant. Growth is resumed also if the fruits are removed. On the other hand, vegetative growth in flower shafts of Composites and Crucifers (**13**) and in the coleoptiles of *Avena* (**12, 5**) and of other cereals is only possible if a growing point or a mass of meristematic tissue is left. If the flower heads (*Compositae*), inflorescence (*Cardamine*) or the meristematic tip (*Avena*) are removed, the growth in length is inhibited.[9] The same is true in wheat. In preliminary experiments in the field in some plants the ear was cut off some time before flowering. The loss of water from the cut was lessened either by putting a paper cap around it or cutting the ear while still in the boot. The growth during the first two days after cutting of the controls was 54 mm. (average of four plants), that of the plants without ear 22.5 mm. (average of five plants), the next two days it was respectively 60 and 7 mm.

If in the experiments just cited the top of a cut flowerstalk or coleoptile was replaced on the stump, growth increased again. There is an abundance of evidence in recent literature (**10, 14,** further literature cited there) that from the growing point or active meristematic tissues a substance diffuses downward, which is usually called a "growth-promoting substance" or "growth hormone," *i.e.*, a substance which makes growth possible. These terms do not imply anything as to its chemical character. If the meristematic tissue is removed, no such substance flows down, thus preventing the growth of cells which otherwise would be capable of increase in size. This is the case in plants from which the apical meristematic tissue (tip of coleoptile, young flowers or ears) has been removed. As soon as fertilization takes place, the embryo and the seed begin to have an independent life and act as independent meristematic tissues. If we assume that the growth-hormones formed inside the developing ovules and fruits do not leach out, we can explain why the growth in length of the flower shaft stops[10] after fertilization; as for the plant, the apical growing point has virtually ceased to exist. The young embryo or embryos form, however, new growth-hormones, which are confined within the fruit, making its growth possible.

[9] The formation of a new physiological tip may complicate this phenomenon (**12, 5**).

[10] In cases of a double growth period, we have to assume a partial leaching out of these substances.

Another hypothesis which has been advanced in order to explain the correlation between growing points and the growth of cells below it (or, on the other hand, the disturbing of this correlation by removing the growing point), is the assumption of a suction force ("Saugkraft") developed by the growing point. In this theory the growing point is supposed to be a center of attraction for foodstuffs (13). The monopolization of nitrogen and carbohydrates by the fruits of tomatoes and cotton (8) is also an example of the attraction or diversion of foodstuffs by the developing fruits from the growing vegetative cells.

These two theories—*viz.*, the theory of the growth-hormones secreted by the growing point and the theory that the foodstuffs are monopolized by the young fruits—are, however, not incompatible, but can be brought together in a more general theory of growth. This is possible if we assume that the growth-hormones have a lyophilic effect upon, or increase the swelling-capacity of the colloids in the protoplasm of the young growing cells, and that they prevent the dehydration of the colloids in the protoplasm of the older cells. As soon as growth-hormones no longer diffuse from the apical growing point down into the vegetative parts below it, as may result from decapitation or fertilization, vegetative growth must stop. But then the imbibition-capacity of the colloids in the older cells must also decrease and water must be lost at this period and later. In this way the cessation of growth in length, the loss of water from the various organs, and the gradual dying of the leaves after flowering can be explained. How the action of the hormones upon the imbibition-capacity of the plasma colloids of a young cell may result in its growth and how this theory places greater importance upon the protoplasm and its hydration than upon the osmotic pressure of the cell sap, I hope soon to show.

Summary

A pure strain of Hard Federation wheat was grown under constant conditions. Ripe (air dry) seeds were obtained in 72 days. Every plant, when full grown, had five leaves and one single stem and ear. The moisture of the different organs throughout the life cycle was determined by harvesting six plants every other day, later on every 4th or 5th day. Sampling errors could be eliminated to a large extent by calculating the moisture and dry weight per unit stem length or per unit leaf area and also by calculating the probable errors.

From the lowest to the uppermost leaf, the dry weight per unit leaf area increases, while the moisture decreases. Throughout the life cycle the dry weight of the leaves increases; the moisture increases until the time of flowering. A sudden drop of moisture occurs in all the organs of the plant 1–2 days after the stamens have appeared. This drop is most pronounced

in the lower leaves, sheaths and internodes. The higher leaves lose less water and are thus able to persist longer than the lower ones. The first sign of this water loss is the yellowing of the tips of the leaves. Therefore the drying of the plant, which leads to the final death of the annual, begins as early as 1–2 days after pollination, *i.e.*, about the time of fertilization.

As an explanation of this loss of water from the different organs of the plant after flowering the theory is advanced that the imbibition-capacity of the plasma colloids, which through the action of the growth-hormones is kept up as long as a vegetative growing point is present, decreases as soon as, through the act of fertilization, this growing point is virtually lost to the vegetative plant.

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NITROGEN METABOLISM IN THE SOY BEAN*

J. E. Webster

Introduction

In attempting to throw light on the protein metabolism of green plants, the variations in the quantities of the simpler water soluble nitrogen compounds of the soy bean (*Glycine max* var. Peking) (8) at various stages in its life cycle were determined and the effects of different conditions of nitrogen supply were studied.

The soy bean was selected for this work, since it is of medium height, is high in nitrogen and matures relatively early.

Emmerling (3) reviewed the earlier literature of this subject very carefully and gives an interesting discussion of his own work with *Vicia faba*. He analyzed plants on nine different dates, making separate analyses of various parts such as the roots, stems, leaves, pods and seeds. He found that the seeds secured their nitrogen at the expense of the entire plant and that the leaves and hulls seemed to act as reserve storage organs for amides. A discussion of the literature on this subject up to a somewhat later date is given by Schulze (10). Sure and Tottingham (13) concluded from a rather complete fractionation of the nitrogen of etiolated plants that α-amino-acids served for the production of amides in the nitrogen metabolism of etiolated plants.

Chibnall (2) has made quite extensive studies on nitrogen metabolism, especially of the leaf of the runner bean. He found that the leaf proteins changed but little after formation and that the fluctuations which occurred in the nitrogen compounds were mainly of the soluble forms. Some question has been raised by Nightingale (5) after his study of protein metabolism in various plants grown under different conditions particularly of light, as to the influence of the soluble forms of nitrogen on plant growth and he suggests that perhaps the ratio of insoluble nitrogen to carbohydrates is one of the most important in plants.

Strowd (12) analyzed the leaves, stems, roots and nodules of soy beans grown under field conditions. He concluded that there was an increase in the nitrate content of the roots during arrested photosynthesis and that nitrates increased in the sap, but not proportionally, when the nitrate supply was increased.

* Investigations pursued at the Ohio State University.

JODIDI (**4**) working with etiolated seedlings of corn followed the decomposition of the proteins in the seedlings by analyzing for various water soluble forms of nitrogen. He finds that there is a steady increase in amide and amino nitrogen and a decrease of peptide nitrogen. However, the increase in amide nitrogen is not proportionally greater than that of the amino nitrogen.

Methods of analysis

Sampling.—The procedure of sampling required about one and one half hours. The leaves with their petioles were removed, weighed and ground. After any cotyledons were removed the stems were cut off at the surface of the soil or sand and weighed and ground. Finally the roots were removed, washed free of adhering soil or sand particles, dried by careful pressing between filter paper, weighed, and ground.

Grinding and extracting.—As soon as weighed, the parts sampled were ground through a Nixtamal mill. Leaves were ground once; stems and roots two or more times depending on the coarseness. This reduced the leaves to a pulp and the stems and roots to a pulp containing shreds of fibrous material.

Before any water was added two separate portions were removed from each sample for moisture and total nitrogen determinations.

The remainder was then stirred with cold distilled water and the watery mass transferred to a filter cloth of closely woven muslin and allowed to drain. After this it was squeezed dry by hand, washed once, placed in a beaker, covered with boiling distilled water and set on a boiling steam bath for two hours. It was then cooled to room temperature and the water extract decanted. The residue on the cloth was washed thoroughly with cold distilled water and squeezed dry. This operation was repeated several times so that the material retained by the cloth and fibrous material was reduced to a minimum.

This method was found to remove practically all of the water soluble forms of nitrogen. Similar results have been secured by TOTTINGHAM, SCHULTZ, and LEPKOVSKY (**15**) who, after a comparison of various types of extraction, concluded that the water extraction method was as satisfactory as any.

Clarifying.—Before the various determinations could be made on this extract it was neecssary to remove the colloidal material. To accomplish this, 50 cc. portions of the well-shaken extract were heated to 65°–70° C. for the leaves and stems, and to 70° or even 75° C. for the roots if the lower temperature did not flocculate the colloidal material. These flocculated samples were then centrifuged until the supernatant liquid was clear, after which they were decanted and the flocculum broken up with 10 cc. portions

of water and centrifuged twice more. Two separate samples were coagulated and then the whole, including washings, was made up to 200 cc.

These extracts, after samples for ammonia and amide determinations had been removed, were preserved with toluene until the amino nitrogen determinations were made.

Moisture.—As previously stated, two samples for moisture determinations were removed at the time of grinding, and placed in paired watch glasses. These, after being weighed, were placed in a vacuum oven which was held constant at 70° C. and through which a continuous small stream of air was drawn. The samples were allowed to remain in the oven for five days after which they were removed, weighed, covered with water and allowed to soak for a day. They were then transferred to Kjeldahl flasks to be used for total nitrogen determination.

Total nitrogen.—The moisture samples after being transferred to Kjeldahl flasks were placed on a steam bath and evaporated to dryness under reduced pressure (**9**). The samples were then analyzed according to the official Gunning method, modified to include the nitrogen of nitrates (**6**).

Soluble nitrogen.—Duplicate determinations were run on 50 cc. portions of the original extract after it had been clarified (see clarifying). These determinations were made in the same manner as the total nitrogen samples above.

Insoluble nitrogen.—This percentage is secured by difference (total–soluble).

Ammonia nitrogen.—For this determination a modified Folin's method was used. Ten cc. samples of the clarified solution, 15 cc. of 52 per cent. K_2CO_3 and 5–8 drops of capryl alcohol were added to the tubes on one side of the Van Slyke-Cullen urea apparatus. To the tubes on the other side were added 10 cc. of N/50 acid, 15 cc. of water, 6 to 8 drops of capryl alcohol and 3 or 4 drops of methyl red indicator. The apparatus was then connected and aerated at such a rate as would draw over 120 liters of water in an hour. The excess acid was then titrated in the tubes, using N/50 alkali.

The results were calculated after subtracting the titration figures from those secured by running blanks. To secure the figures shown in the tables four determinations were made on each sample and the results averaged to get the final figures.

Amide nitrogen.—For these determinations an acid hydrolysis method suggested by Phillips (7) was used. Ten cc. portions of the sample were transferred to tubes of the Van Slyke-Cullen apparatus, several small pieces of pumice and 0.6 cc. of concentrated H_2SO_4 added, and the sample boiled gently under a reflux for two and one-half hours. The samples

were then cooled, nearly neutralized with 2.2 cc. of 20 per cent. NaOH, then cooled again, and capryl alcohol and 20 cc. of 52 per cent. K_2CO_3 added. The system was aerated as in the ammonia determination. Four determinations were made on each sample and the results averaged. The difference between the ammonia titration and the amide titration gives the acid equivalent to the ammonia secured by hydrolysis.

Nitrate nitrogen.—The method used was a modified DEVARDA's alloy method. The method suggested by STROWD (**11**) has been found to be unreliable under certain conditions (**1**) so that a modification by PHILLIPS was used (**7**).

The residue from the amide determination was washed into an 800 cc. Kjeldahl flask using 100–135 cc. of water. This was then boiled nearly to dryness, allowed to cool, one and one-half grams of DEVARDA's alloy added and the whole diluted with 300 cc. of distilled water. The samples were then distilled into N/50 acid containing methyl red as indicator. The excess acid was titrated with N/50 alkali after the samples had been boiled to expel CO_2 and cooled. Blanks were run using 20 cc. of 52 per cent K_2CO_3 instead of the nitrate samples.

Nitrite nitrogen.—The amount present in any case is very small; however, for a rough qualitative test the GRIESS-ILOSVAY method (**14**) was used.

Amino nitrogen.—This determination was made on the cleared solution (see clarifying) using the VAN SLYKE apparatus. No correction was made for the ammonia present.

Other nitrogen.—This percentage was secured by adding together the amino, nitrate, and ammonia results and subtracting this total from the percentage of soluble nitrogen.

Experimental

Due to the large amount of analytical material secured, no attempt will be made to present it all in table form, but only such data as will illustrate the work and show a few of the major points. The summaries for such series as are not represented by tables, were secured by a careful study of the analytical results and of graphs representing the same material. All statements in this paper referring to amounts of the various forms of nitrogen should be understood as refering to percentages of nitrogen in terms of fresh weight of the plant organ analyzed. Also the percentages represent the analyses of at least thirty plants, and in the case of smaller plants, one thousand or more.

SERIES A

HIGH NITROGEN PLANTS GROWN IN SOIL.—These plants were all grown in the greenhouse during the first four months of 1925. This series was con-

tinued for sixteen weeks by the end of which time the plants were mature and the seeds had begun to drop from the pods. The seeds and pods were discarded in all these series. The soil was a rich humus type contained in twelve-inch unglazed pots. The soil had been sterilized before using and at no time were there any nodules observed on the roots. Due to the large number of plants in the pots intended for the first six weeks, a dilute nitrate solution was added to each (one tablespoon of KNO_3 to eight liters of water).

The variations in the percentages of total, insoluble and soluble nitrogen in the leaves, stems and roots are quite comparable with one another with the exception of the soluble percentage in the leaves. The curves for total and soluble nitrogen forms, show two low points; one, two to three weeks after germination when the stored reserve nitrogen seems to be greatly depleted, and before the plant has itself begun to elaborate many nitrogen compounds; the other, at the stage when the seeds are nearly full grown.

The percentages of nearly all forms of nitrogen fluctuate greatly at the sixteenth week when notable degeneration has begun.

The nitrates as a rule are much higher in the stems and roots than in the leaves. The relation between the various parts is in general similar, with the roots showing the greatest discrepancies. The various parts of the plant are lowest in nitrates at about the time of seed maturity (tenth to twelfth week).

Curves for the amino nitrogen run nearly parallel in the case of the leaves and stems but those of the roots diverge widely from the other two. Amino nitrogen is lowest at the inception of visible flowering, and highest at the period when the plants are nearly mature.

There is no striking comparison between the curves for amino and amide nitrogen although it is true that any great variation in the amino is usually reflected in the amide percentage. There is no evidence to show that either the amide or the amino forms of nitrogen are drawn upon for the production of seeds, resulting in a noticeable depletion of that form of nitrogen in any organ, as has been suggested by some writers. The ammonia figures are lowest around the eighth to tenth week and highest during the later weeks when degeneration is greatest. Nitrites are present in the leaves during the earlier stages but their presence in other parts is doubtful.

"Other nitrogen" figures are quite variable and at times show a negative value, possibly due to an incomplete recovery of nitrates in the soluble fraction. There is, however, a remarkable increase in this form in the stems after the tenth week.

SERIES B AND C

HIGH NITROGEN SERIES B AND LOW NITROGEN SERIES C.—These plants were grown in the greenhouse during the months of May, June and July. They were grown in clean quartz sand contained in two-gallon glazed jars which were set in glazed saucers that were kept about one-half full of water. Only distilled water was used in watering plants grown in quartz sand.

The nutrient solution used was made up of the following pure chemicals dissolved in distilled water:

$MgSO_4 \cdot 7H_2O$	2 per cent.
KNO_3	2 per cent.
$Ca(NO_3)_2 \cdot 4HO_2O$	10 per cent.
K_2HPO_4	2 per cent.

For each pot of the high nitrogen plants, 25 cc. of each of the above solutions were mixed with 5 cc. of a 2 per cent. solution of iron citrate and the whole made up to 250 cc. and added weekly.

The solution used for the low nitrogen pots was identical with the high nitrogen one, with the exception that only 5 cc. of the 10 per cent. $Ca(NO_3)_2 \cdot 4H_2O$ was used.

Great difficulty was experienced in keeping green algae out of the saucers and usually after two or three weeks in the greenhouse the saucers were covered with them. The results of the analysis of the high nitrogen series B are shown in tables I, II and III, and of the low nitrogen series C in tables IV, V, and VI.

COMPARISON OF SERIES B AND C.—While a difference in the level of nitrogen supply does markedly influence the physiological appearance of the plants, in this case at least, it does not seem to have greatly affected the growth habits.

The main chemical differences are shown in the considerably lower total nitrogen content of the low nitrogen plants and the presence of nitrites in the stems and roots at only a few periods. One other striking feature of the low nitrogen plants is their very much lower ratio of amide to amino nitrogen as compared to the same ratio in the high nitrogen plants. This statement is true for the leaves, stems, and roots. The percentage of moisture is also slightly higher in the low nitrogen plants.

SERIES D

LOW NITROGEN PLANTS GROWN WITH A NUTRIENT SOLUTION LACKING NITRATES.—These plants were grown in a manner similar to that used in the

TABLE I

PERCENTAGE OF FRESH WEIGHT, LEAVES

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.9781	0.5127	0.4654	0.0815	0.1344	0.0464	0.0185	÷ ÷ ÷ :	0.2310	81.59
2	0.7820	0.5510	0.2310	0.0901	0.0759	0.0147	0.0091	÷ ÷	0.0559	87.44
3	0.7080	0.5301	0.1779	0.0773	0.0610	0.0101	0.0058	÷ ÷	0.0338	87.88
4	0.7342	0.5833	0.1509	0.0299	0.0598	0.0119	0.0054	÷ ÷	0.0558	85.07
5	1.010	0.8527	0.1573	0.0271	0.0602	0.0134	0.0028	÷ ÷	0.0672	84.09
6*	0.8950	0.7045	0.1905	0.0526	0.0745	0.0140	0.0027	÷ ÷ :	0.0607	83.16
8	0.6771	0.5554	0.1217	0.0189	0.0498	0.0093	0.0054	÷	0.0476	82.02
10	1.0007	0.7896	0.2111	0.0261	0.1039	0.0123	0.0138	none	0.0673	75.83

* First blooms were beginning to appear.

TABLE II

PERCENTAGE OF FRESH WEIGHT, STEMS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.5758	0.1583	0.4175	0.0577	0.1912	0.1399	0.0116	faint	0.0630	92.75
2	0.4142	0.0921	0.3221	0.1365	0.1696	0.0579	0.0064	faint	0.0096	91.55
3	0.2595	0.0491	0.2104	0.1374	0.0454	0.0243	0.0030	?	0.0246	90.72
4	0.1880	0.0880	0.1000	0.0416	0.0325	0.0110	0.0072	faint	0.0187	89.11
5	0.2367	0.1441	0.0946	0.0408	0.0468	0.0118	0.0025	÷ ÷	0.0045	88.30
6	0.2341	0.0982	0.1385	0.0764	0.0387	0.0171	trace	÷ ÷	0.0234	85.62
8	0.1765	0.0788	0.0997	0.0212	0.0530	0.0092	0.0043	÷	0.0215	81.99
10	0.3013	0.1599	0.1414	0.0270	0.0680	0.0327	0.0100	none	0.0364	75.03

TABLE III

PERCENTAGE OF FRESH WEIGHT, ROOTS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.2478	0.0772	0.1706	0.0215	0.0842	0.0285	0.0157	÷	0.0492	95.66
2	0.2059	0.0491	0.1568	0.0868	0.0425	0.0069	0.0131	÷	0.0144	95.29
3	0.1795	0.0759	0.1036	0.0631	0.0173	0.0079	0.0094	faint	0.0148	94.54
4	0.1614	0.0821	0.0793	0.0340	0.0212	0.0045	0.0019	faint	0.0222	90.76
5	0.1834	0.1201	0.0633	0.0307	0.0294	0.0027	0.0032	÷	0.0000	91.08
6	0.2069	0.1252	0.0817	0.0456	0.0241	0.0066	0.0027	÷ ÷ ÷	0.0093	90.46
8	0.1526	0.1137	0.0389	0.0203	0.0177	0.0036	0.0015	÷	0.0006	89.03
10	0.1758	0.1044	0.0714	0.0270	0.0247	0.0071	0.0099	faint	0.0098	88.86

TABLE IV

PERCENTAGE OF FRESH WEIGHT, LEAVES

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	1.2006	0.8000	0.4006	0.0364	0.1260	0.0436	0.0164	÷ ÷	0.2218	82.82
2	0.7013	0.5189	0.1824	0.0478	0.0559	0.0142	0.0099	÷ ÷	0.0688	87.42
3	0.6112	0.4632	0.1480	0.0506	0.0337	0.0073	0.0063	÷ ÷	0.0574	88.18
4	0.5612	0.4501	0.1101	0.0242	0.0646	0.0099	0.0056	÷	0.0157	85.66
5	0.6353	0.4977	0.1376	0.0067	0.0704	0.0094	0.0022	÷	0.0583	83.76
6	0.4508	0.3817	0.0691	0.0152	0.0471	0.0063	0.0057	none	0.0011	79.45
8*	0.5146	0.4382	0.0764	0.0104	0.0319	0.0068	0.0026	none	0.0315	81.31
10	0.4703	0.3478	0.1226	0.0176	0.0614	0.0179	0.0081	none	0.0355	77.61

* First blossoms were appearing.

TABLE V

PERCENTAGE OF FRESH WEIGHT, STEMS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.5680	0.1713	0.3967	0.0217	0.1913	0.1359	0.0067	÷	0.1770	92.88
2	....	....	0.2379	0.0916	0.0612	0.0533	0.0071	÷ ÷	0.0780	91.63
3	0.2406	0.0448	0.1958	0.1119	0.0379	0.0200	0.0100	÷	0.0360	90.40
4	0.1577	0.0646	0.0931	0.0386	0.0473	0.0073	0.0044	÷	0.0028	82.29
5	0.1660	0.0768	0.0892	0.0164	0.0574	0.0103	0.0018	none	0.0136	84.58
6	0.1507	0.1100	0.0407	0.0079	0.0458	0.0055	0.0012	none	− 0.0142	76.80
8	0.1377	0.0734	0.0643	0.0192	0.0398	0.0073	0.0079	none	+ 0.0450	88.08
10	0.1748	0.1015	0.0733	0.0345	0.0350	0.0076	0.0106	none	− 0.0068	76.61

TABLE VI

PERCENTAGE OF FRESH WEIGHT, ROOTS

WEEKS	TOTAL N	INSOLUBLE N	SOLUBLE N	NITRATE N	AMINO N	AMIDE N	AMMONIA N	NITRITE N	OTHER N	MOISTURE
1	0.2373	0.0920	0.1453	0.0052	0.0707	0.0332	0.0128	÷ ÷ ÷ ÷	0.0566	95.67
2	0.1188	0.0500	0.0688	0.0279	0.0274	0.0068	0.0056	÷	0.0079	95.54
3	0.1877	0.1107	0.0770	0.0351	0.0172	0.0035	0.0063	faint	0.0184	95.76
4	0.1388	0.0854	0.0534	0.0224	0.0230	0.0027	0.0036	faint	0.0044	92.45
5	0.1442	0.0949	0.0493	0.0105	0.0298	0.0066	trace	faint	0.0090	92.08
6	0.1457	0.0896	0.0561	0.0060	0.0145	0.0012	0.0023	faint	0.0333	90.60
8	0.1330	0.0688	0.0642	0.0169	0.0189	0.0028	0.0031	none	0.0252	80.61
10	0.1315	0.0799	0.0516	0.0196	0.0231	0.0041	0.0053	none	0.0036	89.98

two preceding series and differed only in the use of the following minus-nitrate nutrient solution:

$MgSO_4.7\,H_2O$	2 per cent.
KCl	2 per cent.
$CaCl_2.2\,H_2O$	4 per cent.
K_2HPO_4	2 per cent.

Samples were taken when the plants were two, four and six weeks old. The analyses of this series show but one significant fact; namely, that there is a constant decrease of nitrogen in all parts of the plant and that this decrease is shared alike by all of the forms for which analyses were made.

Series E

Low nitrogen plants grown in quartz sand to which no nutrient solutions were added.—The plants of this series were grown during July and August in quartz sand prepared in the same manner as that in B and C. These, however, were not given any nutrient solution and consequently were dependent upon the material stored in their cotyledons for growth.

The total and soluble nitrogen curves for the leaves, stems and roots show very much the same fluctuations, there being, after the second week, a small continual fall in each. The insoluble nitrogen, however, is quite variable. Points of particular interest are the increase in the amino, amide, and ammonia forms of nitrogen at the second week and the small change in the insoluble fraction at all times in the leaves and stems.

The various soluble forms with the exception of the amino nitrogen at the sixth week show somewhat of a drop after the second week.

The trace of nitrites present in the various parts at the end of the first week is interesting, although no explanation of their presence can be given.

It is also worth noting that compared with series D, in which the percentages of all forms fall off rapidly in the leaves and stems, there is only a slow decrease, in these organs, in the percentage of total nitrogen; and this decrease occurs almost entirely in the soluble fraction. In the roots, however, the decrease is shown equally in both fractions.

Summary

Series A presents data for plants grown in humus soil, in pots in the greenhouse. These plants were sampled weekly. Series B and C were grown in sand cultures in the greenhouse and differ only in the amounts of nitrates applied, series B having received the larger amount. Series D and E were grown similarly to B and C and differ only in the type of nutrient solution supplied; D contained no nitrates and E received only distilled water.

In series A, B and C there is a weekly variation of percentages of total nitrogen in no particular direction. This would not be taken into account by data representing only one or two samplings during the life cycle of the plant and seems to be a point that has been overlooked by many in interpreting chemical analyses of plants.

The fact that the supply of nitrogen may be varied over such wide limits as shown in series B and C and yet not seriously disarrange the growth cycle is of interest in discussing the carbohydrate-nitrogen ratio. Data concerning the carbohydrate fluctuations would, however, be necessary before this could be discussed fully.

From a consideration of the first series, A, and some data unpublished it seems that the addition of nitrates results in a temporary increase in nitrate storage in all organs of the plant. This is soon, however, reduced to normal without any noticeable increase in the other soluble forms.

It is worth noting that the soluble nitrogen is relatively much more abundant in the stems and roots than in the leaves of all the plants for which data are presented.

There is no evidence to show that, under these conditions, there is any tendency for any of the forms of nitrogen here considered to become grouped in a particular organ of the plant with a resultant loss to any other organ.

It must be borne in mind that in considering these data the plants were grown under greenhouse conditions and this may account for some of the discrepancies between this work and that reported for some field work by other investigators.

On the basis of the amount present, amide forms of nitrogen do not seem to be of any more importance in nitrogen metabolism of plants than are the amino-acids. There does, however, seem to be a tendency for the ratio of amide to amino nitrogen to increase when a large supply of nitrogen is available to the plant. This might be interpreted as showing that amide forms occur as soluble storage forms.

It seems possible that the formation of nitrites may represent an intermediate stage in nitrogen metabolism when nitrates are supplied to plants. On the other hand, nitrites do not seem to occur in detectable amounts when organic forms of nitrogen are used or when plants are living on their stored reserves.

Although the percentages of ammonia are very small, in each series there seems to be reason for concluding that ammonia is one of the intermediate stages in both the synthesis and destruction of proteins in plants.

The column marked "Other nitrogen" represents a nearly unknown fraction of soluble nitrogen. It is hoped, when time is available, to carry on work leading to a fraction of this portion.

Conclusions

1. In general, the variations of the various types of nitrogen compounds in the roots, leaves and stems of any particular series are quite similar.

2. There is little or no evidence to support the idea that the leaves, stems, or if present, the seeds, draw upon the nitrogen reserves of the roots.

3. Amino-acids having amide groupings, on the basis of the amount present, are of no more importance in protein metabolism in plants than other amino-acids, although there is some evidence to show that amide nitrogen may be one of the most usual soluble storage forms.

4. Additional proof has been found for the idea that ammonia is the first and last product of nitrogen metabolism in plants.

5. There is considerable daily fluctuation of the various forms of nitrogen in no uniform direction.

6. Within a wide range, when the external nitrogen supply is the only variable, only the general appearance and not the physiological cycle of the soybean is changed.

The writer wishes to express his indebtedness to Dr. T. G. Phillips for suggesting the problem and the helpful criticism he has given; to Dr. R. C. Burrell for his suggestions and criticisms; and to Dr. William Crocker for the aid and use of the equipment of the Boyce Thompson Institute for Plant Research where the analytical part of this problem was carried out.

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THE EFFECT OF ACCUMULATED CARBON DIOXIDE ON PLANT RESPIRATION*

J. J. Willaman and J. H. Beaumont

In a study of the biochemical basis of winter hardiness in apple trees, the writers have had occasion to measure the relative rates of respiration of tender and hardy varieties. In the first series of measurements (**4**) continuous aspiration was employed; that is, during the period of measurement CO_2–free air was continuously passed through the chamber containing the twigs.

Because of certain mechanical difficulties in this method over long periods, discontinuous aspiration was then tried; that is, the CO_2 was allowed to accumulate in the chamber and was measured at the end of the period. It became apparent that an entirely different picture of respiratory activity was obtained by this procedure. Therefore, a more detailed study of the phenomenon was undertaken, the results of which are presented in this paper.

As long ago as 1881 Müntz (**16**) reported that grain emits many times as much CO_2 when it has access to fresh air as when it is confined in a container. Mangin (**15**) in 1896 pointed out that the respiration of germinating seeds is decreased by the presence of 5 per cent. of CO_2, and that the respiratory quotient becomes greater. Czapek, in his excellent review of plant respiration (**12**), does not discuss this particular phase of it. Many studies on respiration have been conducted by both methods. The well-known Osterhout method (**19**) is of the continuous type. Bailey and Gurjar (**1, 3**) used the discontinuous method on grains, conceding that it indicated a rate that continually decreased with time. Spoehr and McGee (**23**) described quite definitely the effect of changing plants from one concentration of CO_2 to another, which in effect is really the procedure in the discontinuous method. Olney (**18**) used the latter method on bananas, while Bergman (**6**) employed the continuous on cranberry plants. The present writers used the continuous method in the work on apple twigs reported preliminarily (**4**). Thomas (**25**) has found that concentrations of CO_2 above 12 per cent. in the air surrounding apples tend to increase the production of ethyl alcohol and of acetaldehyde; in other words, to change the respiration to a zymasic type.

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In the light of the facts briefly reviewed above, it is evident that the accumulation of CO_2 in the atmosphere surrounding plant tissue has an appreciable effect on the respiration of that tissue.

Experiments with apple twigs

In the work reported here the twigs were gathered during the first two weeks in March, 1926. Only the one-year-old wood was used. The cut ends were paraffined. The twigs were exposed to room temperature for only a few minutes. From 150 to 200 gm. of the twigs were packed into glass tubes about 5 cm. in diameter and about 45 cm. long, and the tubes placed in the thermostat immediately. The temperature was 0° C. in these

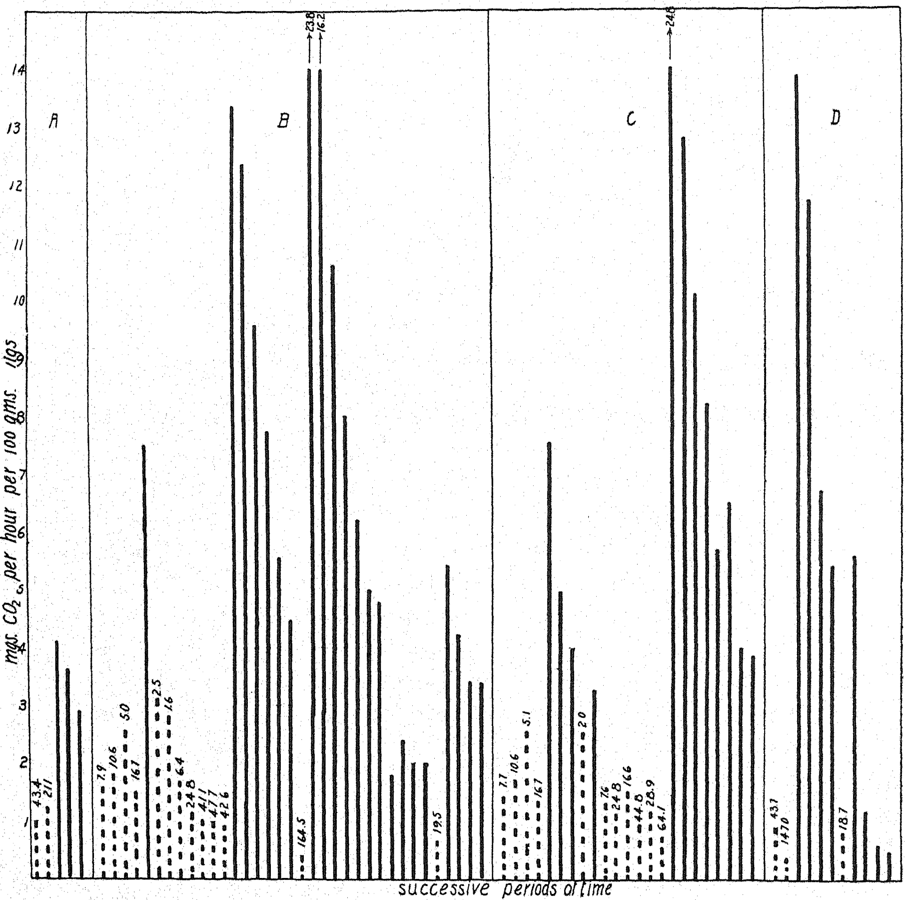

FIG. 1. Respiration of apple twigs at 0° C. The dotted line represents the rate during a period when the CO_2 is allowed to accumulate; the solid line represents a 20-minute period of continuous removal of CO_2. A, B, C, and D are different lots of twigs.

experiments. The thermostat, controls, air washing and circulating devices, and absorption towers were the same as described elsewhere (5).

The data are presented in figure 1. The vertical axis represents the rate of CO_2 production, expressed as mg. of CO_2 per hour per 100 gm. of twigs. The horizontal axis represents successive periods of time. The dotted lines stand for periods during which the CO_2 was allowed to accumulate, followed by a 20-minute period of aspiration to remove the CO_2. The lengths of these periods in hours is indicated by the number at the end of the dotted line. The solid line represents a 30-minute period, 20 minutes of which were consumed in aspiration, and 10 minutes in changing apparatus in preparation for the next period of aspiration. These half-hour periods thus represent periods of practically continuous aspiration. The time required for complete removal of CO_2 in the chamber, was, of course, carefully determined, and found to be about 12 minutes; 20 minutes were then adopted for safety. The object of this schedule was to measure the rate of CO_2 emission during varying periods by the discontinuous principle, followed by its measurement with continuous aspiration. Sections A to D represent different lots of twigs.

It will be seen that each dotted line or each series of them, is followed by a series of solid lines, each succeeding one of the latter being shorter. This means that a period in which the CO_2 is allowed to accumulate in the chamber is followed immediately by a much higher rate of respiration. The rate gradually subsides, but in the one case in lot B where a constant rate was attained, it was after about 35 hours.

There is some evidence that the magnitude of this phenomenon is proportional to the amount of CO_2 that has accumulated in the chamber, or in other words, the length of time of this accumulation. In lot C it is slightly evident after a 2-hour period. It is most pronounced following the 164.5 hour run in B and the 64.1 hour run in C. Seven periods of moderate length in B are as effective as two periods of much greater length in D.

It is quite evident that from these data, it would be impossible to say what is the normal rate of respiration of these twigs. Continuous removal of the CO_2 is imperative.

Casual study of the data for the accumulation periods indicates that in general the rate for the period is inversely proportional to the length of the period: in other words, that the rate continuously decreases with time. In order to bring out this relation more clearly, the amount of CO_2 for each period was plotted against the length of the period. The resultant graph is shown in figure 2.

A curve was fitted to these points. This curve corresponds to a logarithmic one expressed by the formula:

$$\frac{CO_2}{\log t - 0.566} = k$$

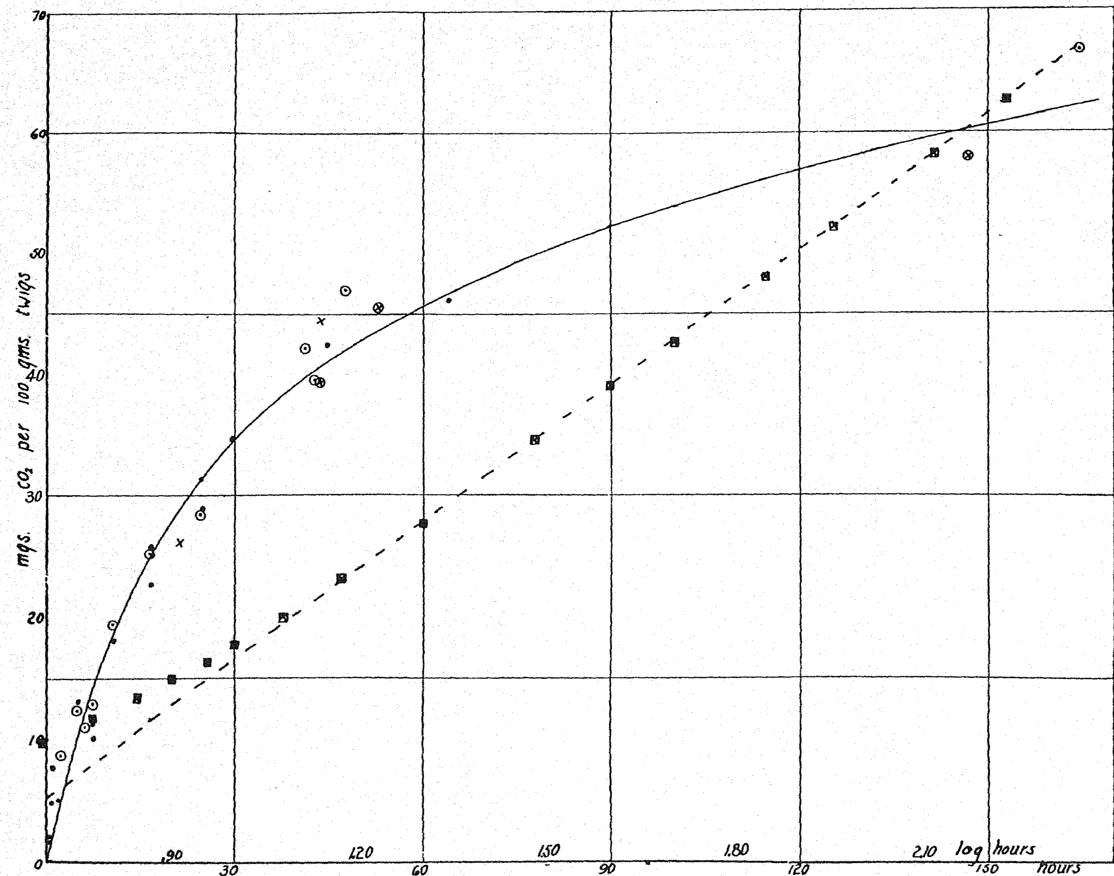

FIG. 2. Respiration of apple twigs at 0° C. as determined by the accumulation method. The symbols along the solid line represent different lots of twigs, with the CO_2 plotted against time in hours. The dotted line represents CO_2 plotted against log of time, using points on the solid line.

The solid line is CO_2 against t in hours, while the dotted line is CO_2 against $\log t$. Table I gives the values of k for certain values of t.

TABLE I

VALUES OF k FOR THE RESPIRATION OF APPLE TWIGS ACCORDING TO THE CURVE IN FIGURE 2

t	CO_2	$\frac{CO_2}{\log t - 0.566}$
hours	mg.	k
5	10.0	75.2
10	17.7	40.8
20	28.0	38.1
30	34.6	38.0
40	39.3	37.9
50	42.7	37.7
70	48.2	37.7
90	52.2	37.8
100	53.9	37.6
130	58.3	37.7
170	62.7	37.7

The curve is in satisfactory agreement with the experimental values. The log-time curve is a straight line beyond the 30- or 40-hour period. During the shorter periods it is not a straight line because the respiration values are affected by the high temporary rates at the beginning of each period, in accordance with the data of figure 1 previously discussed. If the short period measurements had followed in all cases periods of continuous aspiration, during which the rate of CO_2 emission had attained a constant value, these short period measurements would no doubt have conformed much more closely to the equation.

This fact emphasizes the necessity, in plant respiration work, of taking cognizance of the history of the material during the period immediately preceding that of the measurement.

Experiments with potato tubers

Small potato tubers which would just enter the respiration tube were used in the same type of experiments. The tube was kept on the laboratory table at room temperature without special control. Any change in temperature during the continuous phase of aspiration was guarded against, and this is the only place that small changes in temperature could change the present results.

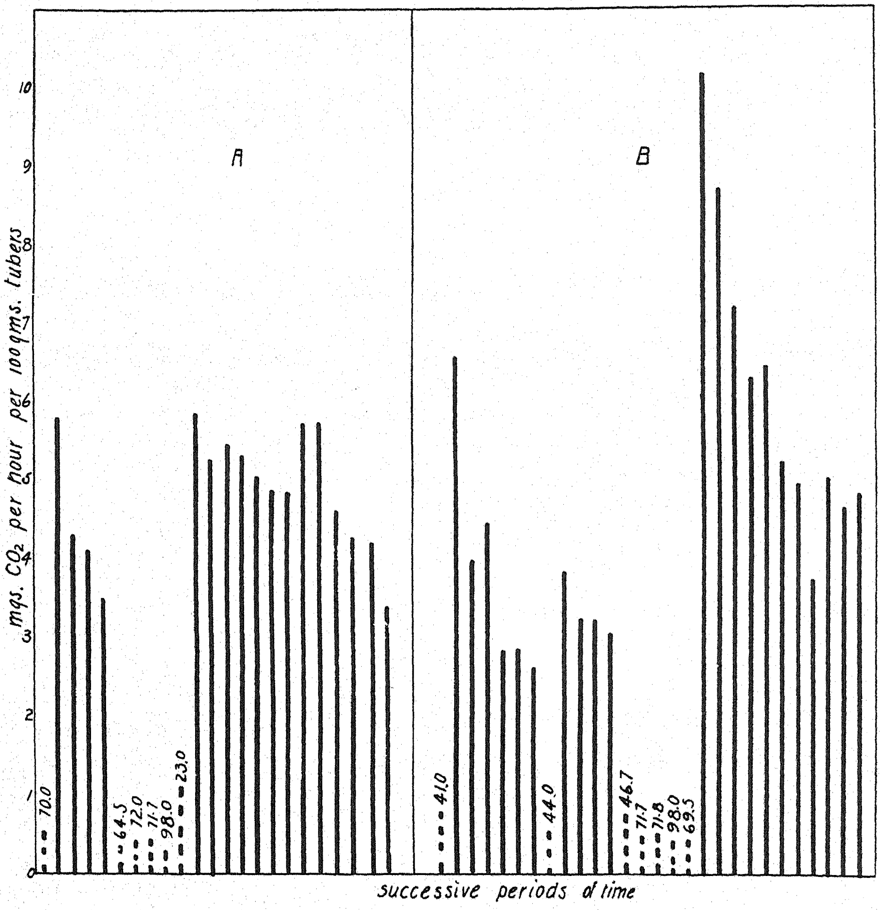

FIG. 3. Respiration of potato tubers at room temperature. See fig. 1 for description of conventions.

The results with two lots of tubers are shown in figure 3. The effect of accumulated CO_2 on the subsequent rate of its production is practically the same as in the case of the twigs.

Experiments with wheat

Since the results of BAILEY and GURJAR (**3**) indicate that the respiration of wheat grain may be affected by accumulated CO_2 in a manner similar to apple twigs, a series of experiments was run to determine this effect. Sound Marquis wheat was tempered to contain about 16 per cent. moisture. Respiration tubes like the ones used for twigs were filled with the wheat, about

500 gm. being required. The tubes were placed in a water thermostat at 40° C. This relatively high temperature was used because of the much slower rate of CO_2 production of this material in comparison with twigs.

The results are presented in figures 3, 4 and 5. In these runs the 20

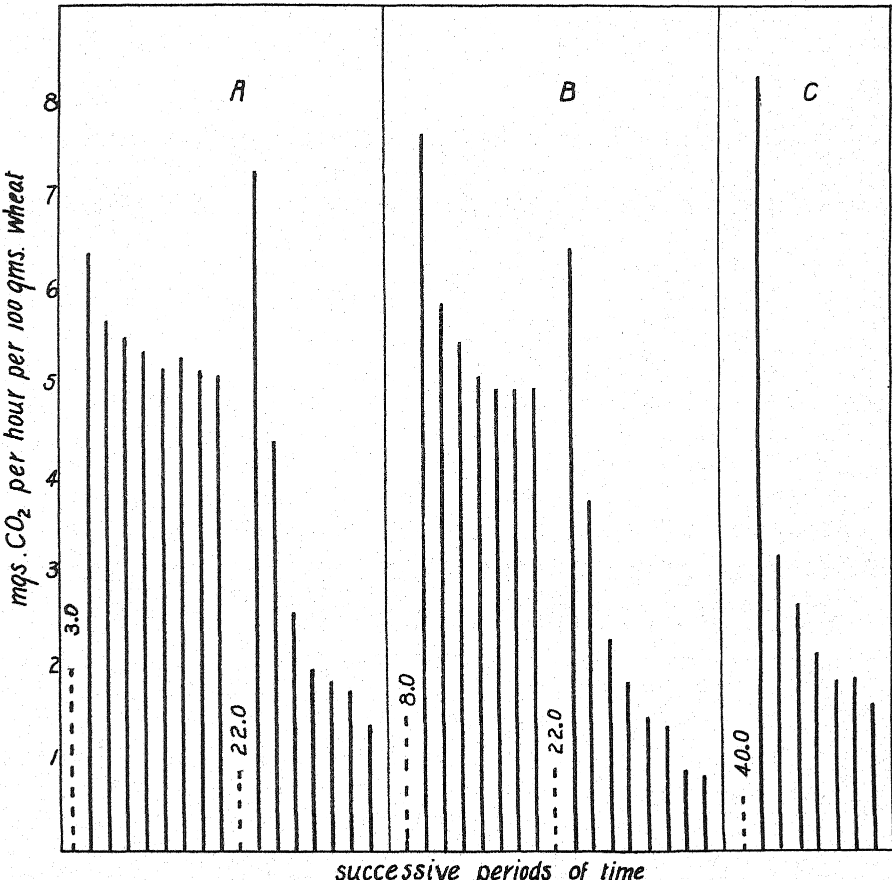

FIG. 4. Respiration of wheat at 40° C. See fig. 1 for description of conventions.

minutes of aspiration was followed immediately by a second aspiration. Therefore the solid lines represent 20-minute periods instead of 30. Practically the same picture is obtained here as in the case of the twigs and of the tubers. Each period of accumulation is followed immediately by an increased rate of CO_2 production. The rate gradually subsides, probably becoming constant after two or three hours, although this was not attained in any of these runs.

A possible explanation of the CO_2 effect

It has been shown that three types of plant tissue, twigs, tubers and wheat grain, exhibit the same reaction to the accumulation of CO_2 in the surrounding atmosphere. For the sake of clearness, this reaction may be described again: When the CO_2 is allowed to accumulate, the rate of production of the CO_2 diminishes in a logarithmic ratio. In the case of twigs, at least, the amount of CO_2 produced is proportional to the logarithm of time. When accumulated CO_2 is removed, the rate of its production immediately assumes a far higher value; and the magnitude of this increased value is possibly proportional to the amount of CO_2 previously accumulated. It is a matter of several hours before the rate attains a constant value.

This is in strict accord with the conclusions of SPOEHR and McGEE (**23**): "When the CO_2-content of the air surrounding a leaf is changed from a lower to a higher concentration, the leaf shows a reduced rate of CO_2 emission for a period following the change, then increases, and finally again attains about the same rate as before the change in CO_2-content was made. Conversely, when the CO_2 content of the air surrounding a leaf is changed from a higher to a lower concentration, the leaf shows a primary increased rate of CO_2-emission and subsequent decrease to the original rate."

Such being the facts, we are of course interested in attempting an explanation. One explanation is that we are observing merely an equilibrium between the CO_2 in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess CO_2 in the latter is removed but slowly when aspiration is commenced.

Another possible explanation was suggested by Dr. R. A. GORTNER. It is that the accumulation of CO_2 in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point, and hence increases its permeability, which is responsible (perhaps through increased enzyme activity) for an actual increased rate of CO_2 production. The increase would probably be merely potential as long as there was a high content of CO_2 in the air surrounding the tissues, but would become actual as soon as aspiration was commenced.

It will be necessary to review the existing evidence in favor of such a proposition before presenting the experiments designed to demonstrate it.

That the membranes would be more permeable if their proteins were nearer the isoelectric point is possibly illustrated by the experiments of HITCHCOCK (**13**). We can best quote from him directly: "The permeability of gelatin-coated collodion membranes, as measured by the flow of water or of dilute solutions through the membranes, has been found to vary with the pH of the solutions. The permeability is greatest near the isoelectric point of the protein; with increasing concentration of either acid or alkali it

decreases, passes through a minimum, and then increases. These variations with pH are qualitatively in accord with the assumption that they are due to swelling of the gelatin in the pores of the membrane, the effects of pH being similar to those observed by LOEB on the swelling of gelatin granules. Indications have been found of a similar variable permeability in the case of membranes coated with egg albumin, edestin, serum euglobulin, and serum albumin."

That the acidity of the tissue fluids is increased by the accumulation of CO_2 is well known, and does not need a specific illustration. That such an increase in acidity would bring the proteins nearer to their isoelectric points is, however, not so easy to argue from existing data. The isoelectric point of but few plant proteins has been determined. CHIBNALL (**8, 9, 10, 11**) has isolated glutelins from the leaves of spinach, corn, alfalfa and other plants, and has found them to have very similar properties, with an isoelectric zone of pH 4.0 to 5.0. In all cases the reaction of the expressed sap was alkaline with respect to the isoelectric point of the protein. The gliadin of the wheat berry has an isoelectric point of 6.6 according to ETO, and 5.76 according to HOFFMAN and GORTNER (**2**, p. 245); that of the glutenin is 6.8 to 7.0 (**2**, p. 250); and that of the leucosin is 4.6 (**2**, p. 253). ROBBINS (**20, 21, 22**) has determined what he believes to be the isoelectric point for tissues, and has obtained the following values: potato tuber, 6.0 to 6.4; mycelium of *Rhizopus nigricans,* 5.0; that of *Fusarium lycopersici,* 5.5; that of *F. oxysporum,* 4.9; that of *Gibberella saubinetii,* 6.2; soy bean root tips, 6.2 to 6.4.

The most careful measurements of the pH of cell sap are those by NEEDHAM and NEEDHAM (**17**) and by CHAMBERS and POLLACK (**7**), in which experiments indicator dyes are introduced into the cell by micrurgical technique. The former obtained values of 6.6 for various marine ova, and the latter 6.6 to 6.8 for starfish eggs. When the cell is injured, as by a tear, the pH rapidly diminishes to pH 5.4 to 5.6. If the latter phenomenon holds also for plant cells, the conclusion is that the host of measurements of the pH of expressed sap indicate a higher acidity than actually obtains in the normal tissue. Most of such expressed saps show a pH of 5.0 to 7.0.

Taking a general survey of the data reviewed above, it is fair to conclude that there is some indication that plant sap is alkaline with respect to the isoelectric point of its proteins. CHIBNALL has the only direct evidence of this. If this relation be assumed, the conclusions would follow that CO_2 could bring the reaction nearer to the isoelectric point of the proteins, that this would increase the permeability of the protoplasm, and that more rapid respiration could result.

A method used in the attempt to obtain more or less direct evidence of this chain of events was to introduce HCl gas, for short periods, into the

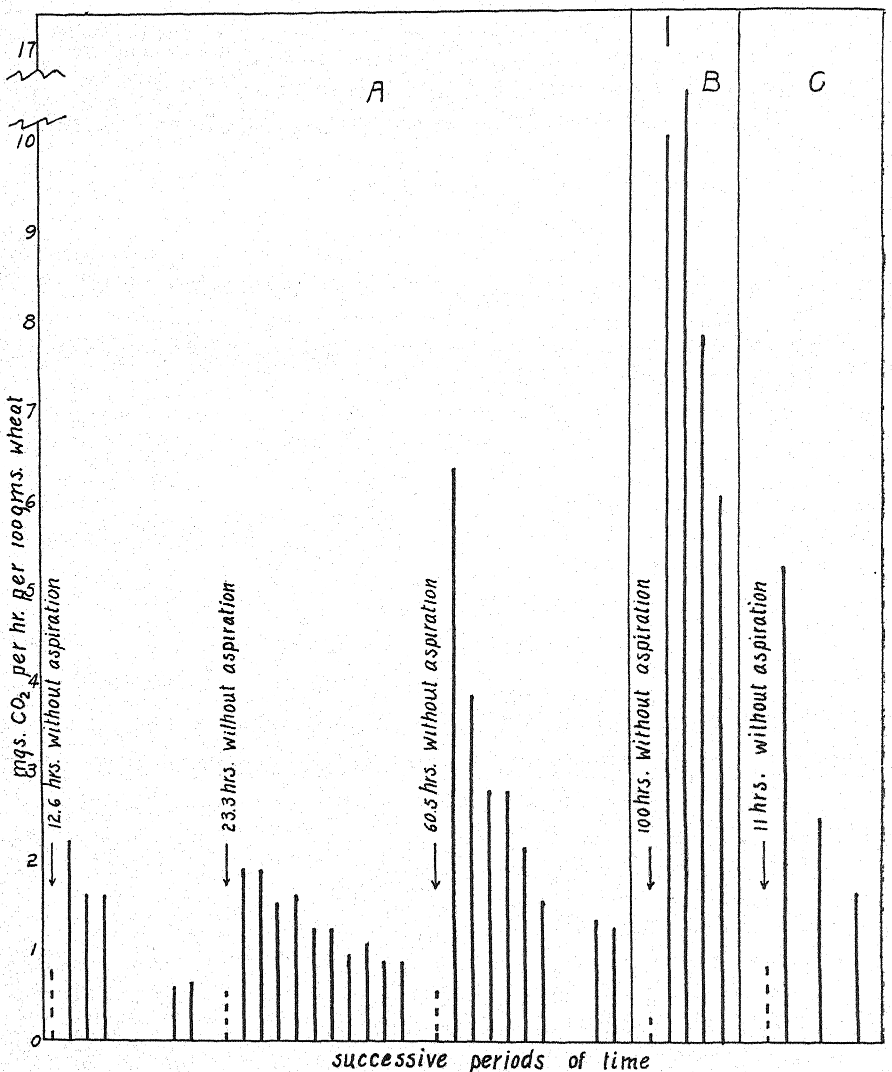

FIG. 5. Respiration of wheat at 38 to 42° C. See fig. 1 for description of conventions.

respiration chamber containing wheat and to observe whether this increased the rate of emission of CO_2. This has been done, with fairly positive results in favor of the above hypothesis.

The air entering the chamber was first bubbled through a solution of HCl for 30 minutes in one run, and for 1.5 hours in the other. The chamber then remained for one hour without aspiration. Then the aspiration was continued and the CO_2 determined. The titration procedure was modified

so as to allow for any HCl that might accompany the CO_2. HNO_3 was used for titrating instead of the usual HCl; $Ba(NO_3)_2$ was used to precipitate the carbonates instead of $BaCl_2$; and at the end of the acid titration the chlorides were titrated with silver nitrate. Only a few mg. of HCl reached the absorption tower.

The results of the two experiments are given in figure 6. In the trial run shown in the upper portion of the graph, aspiration was continued for one hour so as to establish the rate of CO_2 production. Then the air was bubbled through concentrated HCl for 30 minutes. After standing for one hour, the accumulated CO_2 was measured, and is represented by the dotted line in C. Following this there is seen to be the usual increase in rate, followed by a decrease to a level which is somewhat lower than the rate in A. Another one-hour period of standing (D) was followed by another but slighter increase.

In the second run, in the lower half of figure 5, a control period without HCl was measured first. This involved a cycle of three periods (A, B, C). Following this was the treatment with HCl as indicated in the chart, and the measurement of the subsequent rate of CO_2 production. Period C, following the control, is characterized by an initial rate of 3.5 mg. Period F, following the HCl treatment, is characterized by an initial rate of 5.4 mg.

The writers are convinced that the HCl treatment has duplicated the effects of accumulated CO_2. To be sure, the wheat was sooner or later injured by the treatment, but it was not visible during the course of the runs, and in any event the increase in CO_2 production is a fact.

Another line of evidence that disfavors the idea of mere solution of CO_2 in the tissues is the size of the three tissues used. It is reasonable to expect that the thicker the tissue, the slower will be the rate of diffusion of the dissolved CO_2 into the surrounding air. In the three tissues used the time required to attain a constant value should decrease in the order, wheat, twigs, tubers. There is no evidence of this differential rate in the charts.

The writers do not believe that the exhaustion of oxygen from the chamber is a deciding factor. In the case of the twigs and the tubers, the oxygen was never exhausted; in the case of the wheat it was exhausted only during a few long runs. Furthermore KARLSON (**14**) states: "The effects of ether, benzene, and alcohol on the aerobic and anaerobic production of CO_2 by wheat (seedlings) are closely similar. This would seem to indicate that the fundamental processes or the master reactions on which they depend are similar."

On the basis of these experimental findings and of the suggestive evidence of the pH values found in the literature, the writers are led to adopt

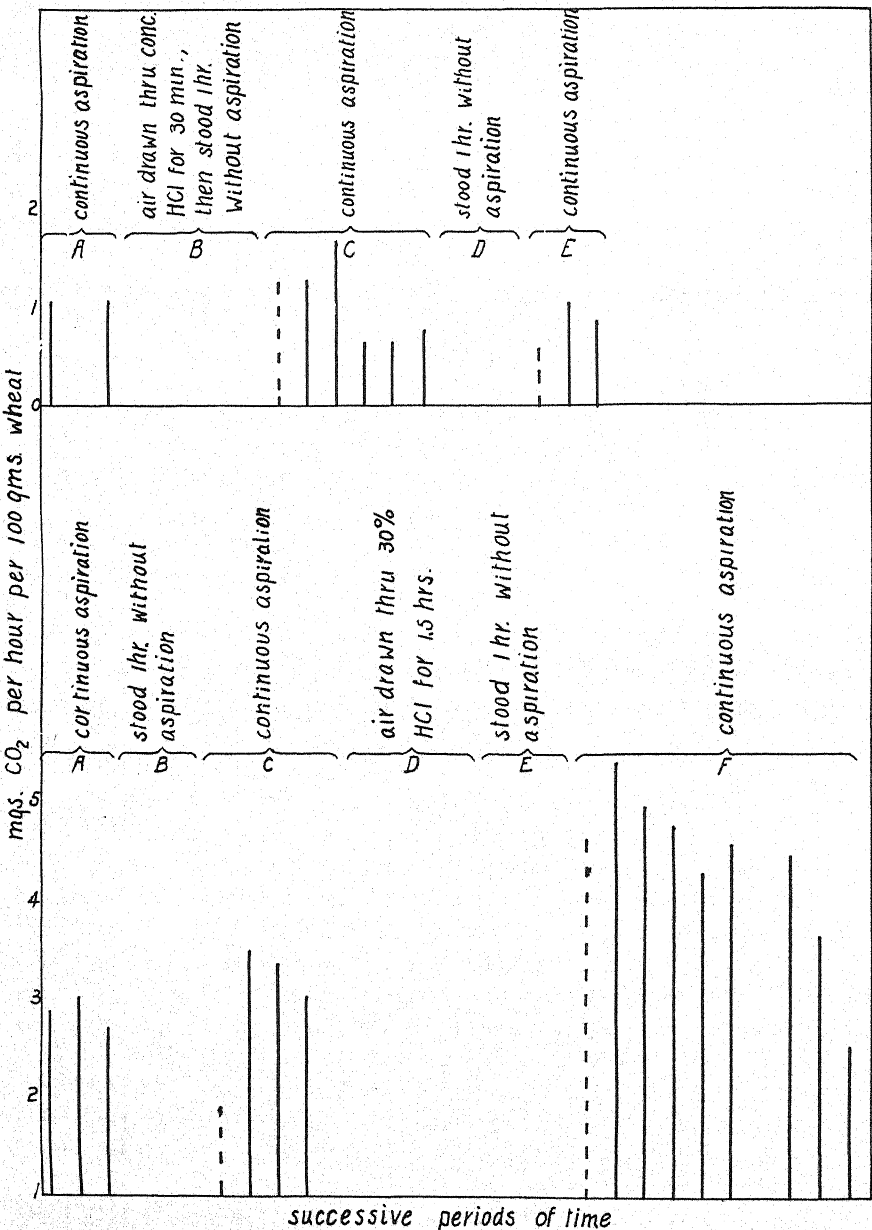

FIG. 6. Respiration of wheat at 40° C. as affected by HCl gas.

the explanation here outlined for the effect of accumulated CO_2 on the rate of respiration of plant tissues.

Conclusions

The respiration of apple twigs at 0° C., of potato tubers at about 22° C., and of wheat grain at 40° C. has been studied from the standpoint of the effect of allowing the CO_2 to accumulate in the respiration chamber. Under such conditions the rate of CO_2 production continuously decreases with time. After the first 30 or 40 hours the relation is expressed by the formula

$$\frac{CO_2}{\log\ t - 0.566} = k.$$

During the first 30 or 40 hours the rate is affected by a phenomenon which can be described as follows: When aspiration of the atmosphere surrounding the tissue is commenced, after a period of accumulation of CO_2, the rate of respiration immediately assumes a far higher value than it had during the accumulation period. The magnitude of this value is possibly proportional to the amount of CO_2 previously accumulated. It is a matter of several hours before the rate attains a constant value.

One explanation of this phenomenon is that we are merely observing an equilibrium between the CO_2 in the atmosphere surrounding the tissues and that which is dissolved in the tissues; and that the excess CO_2 in the latter is removed but slowly when aspiration is commenced.

Another possible explanation is that the accumulation of CO_2 in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point and hence increases the permeability of the protoplasm; and that this is responsible for an actual increase in rate of CO_2 production. The evidence in the literature on the pH of cell sap and on the isoelectric points of plant proteins bears out this view to a certain extent. Direct evidence in its favor was secured by passing HCl gas into a respiration chamber containing wheat grain. A duplicate of the CO_2 effect was obtained.

Although admitting that the proof for the latter explanation is still far from complete, the writers nevertheless subscribe to it, and offer it for the criticism of others.

The conclusion should be emphasized that the investigator should take cognizance of the CO_2 effect in deciding which procedure, the continuous or the discontinuous, to adopt for the work in hand. Under some circumstances, of course, as in a study of grain and fruit in storage, more useful knowledge might be secured by the accumulation method.

The writers wish to acknowledge the help of Mr. Leo M. Greene in securing some of the data in this paper.

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ON THE NITROGEN CONTENT OF GROWING CULTURES OF *MYCODERMA* AND OF *SACCHAROMYCES CEREVISIAE*

LEO M. CHRISTENSEN

Introduction

In a previous communication by FULMER and CHRISTENSEN (5) it has been shown that the nitrogen content of a growing culture of an organism designated as "yeast no. 12" is a function of time and of pH as determined by the Kjeldahl method. It was also noted that the cultures showed a preliminary loss in nitrogen followed by a gain and that the greatest gain took place at the pH at which there was the antecedent maximum loss. It was suggested that the loss may have been more apparent than real, the hypothesis being that the nitrogenous compounds may have undergone a change rendering them unanalyzable by the regular Kjeldahl method. This led to the development of a modified method, utilizing a preliminary oxidation with hydrogen peroxide in dilute sulphuric acid solution. This method which gives higher yields of nitrogen for yeast than the usual Kjeldahl method has been described in detail by CHRISTENSEN and FULMER (2).

The data presented in this communication deal with the change in nitrogen content of growing cultures of the organism previously studied and of *Saccharomyces cerevisiae* as determined by the two analytical methods, the Kjeldahl and the modified method previously mentioned.

Organisms used

Two types of organisms have been used in our studies of yeast nutrition, both of which were isolated from a cake of Fleischmann yeast and designated by us as yeasts nos. 11* and 12* (1). These two types resemble those described by EDDY, KERR and WILLIAMS (3). Number 11 is *Saccharomyces cerevisiae*. On three different occasions within the course of a year no. 12 was found to compose about 2 per cent. of the organisms in the commercial product. This type was isolated from the same source by MACDONALD (7) and classified as a *Mycoderma* by this author. A careful study of the organism in this laboratory verifies this classification.

The properties of the two organisms are given in table I.

* These organisms are listed in the American type culture collection as no. 4226 (*Saccharomyces cerevisiae*) and no. 4225 (*Mycoderma*) respectively.

TABLE I

PROPERTIES OF ORGANISMS COMPARED IN VARIOUS MEDIA

PROPERTIES OBSERVED	SACCHAROMYCES CEREVISIAE	YEAST NO. 12
Colonies on 2 per cent. glucose, 0.5 per cent. peptone agar		
Growth	Slow	Rapid
Form of Colony	Circular	Circular
Surface	Smooth, moist	Rough, dry
Elevation	Convex	Convex
Edge	Entire	Filamentous
Internal structure	Amorphous	Granular
Branching	Absent	Predominant
Shape and size of cells	Spherical or ellipsoidal, 6–8 μ diam.	Elongated, 8–20 μ x 2–4 μ
Size of colony 48 hours	1 mm. diameter	4 mm. diameter
Growth in medium E,* inoculated from colony on glucose-peptone-agar		
48 hours	Growth	Visible growth
72 hours	Count 89.5	Count 1.0
	Cells spherical, little branching	Cells spherical, little branching
Growth in medium K,† inoculated from colony on glucose-peptone-agar		
48 hours	No growth	Visible growth
72 hours	No growth	Count 23.0
Fermentation of carbohydrates. (Peptone 0.5 per cent., carbohydrate 2.0 per cent.)		
Glucose	Gas and alcohol	Gas
Levulose	Gas and alcohol	Gas
Sucrose	Gas and alcohol	Small amount of gas
Maltose	Gas and alcohol	Gas
		Esters rather than alcohol are formed
Growth in wort		
24 hours	Foam	Heavy froth
48 hours	Count 620	Count 3380
	Bottom growth, cells spherical, 6–8 μ diameter, no branching	Dry, wrinkled surface growth. Cells elongated, 5 x 7 μ, branching predominant
Spore formation 10 day growth on carrot infusion-$CaSO_4$-agar		
	Typical ascospores of *S. cerevisiae*	No spores

* Medium E contained per 100 cc. 0.10 g. K_2HPO_4; 0.10 g. $CaCl_2$; 0.04 g. $CaCo_3$; 0.188 g. NH_4Cl; 2 g. sucrose after FULMER, NELSON and SHERWOOD (6).

† Medium K contained per 100 cc. of medium 0.10 g. K_2HPO_4; 0.002 g. NH_4Cl; 2 g. sucrose.

It is apparent that the *Mycoderma* grows more poorly in medium E than the yeast, while the reverse is true for the medium low in nitrogen. Several organisms kindly furnished by F. W. TANNER were grown on medium K. All of these organisms except *Saccharomyces cerevisiae* had a tendency to grow on the surface, a characteristic which would seem desirable under conditions in which nitrogen is taken from the air. The organisms so tested and the summary of results are listed in table II.

TABLE II

GROWTH ON MEDIUM K OF SEVERAL ORGANISMS

ORGANISM	GROWTH
No. 12 (*Mycoderma*)	++++
Willia saturnus	++++
Torula humicola	+++
Willia anomala	++
Saccharomyces chevalieri	++
Mycoderma vini	++
Tichia membrane faciam	+
Saccharomyces anomalus	+
Saccharomyces neoformans	+
Saccharomyces hominis	+
Saccharomyces cerevisiae	+

There is evidently a considerable difference in the ability of these organisms to grow in a low nitrogen environment. In the following studies the first (*Mycoderma*) and last organisms (*Saccharomyces cerevisiae*) were employed.

The media employed

In omitting the ammonium chloride from the medium, not only was the organism deprived of a good source of nitrogen but likewise of the physicochemical effect of the ammonium chloride. This rôle has been emphasized by FULMER and co-workers (**4, 6**). The growth of the *Mycoderma* in the synthetic media tested was not large enough to permit consistent quantitative estimation of changes in nitrogen content of the medium. It seemed advisable then to add the growth stimulant, bios, in amounts permitting sufficient growth for analysis and with the minimum addition of nitrogen. For this purpose high-grade molasses, one sample containing 0.21 per cent. nitrogen (Kjeldahl) and the other 0.41 per cent. nitrogen, was used.

The medium contained per 100 cc. 6 g. molasses and 0.50 g. K_2HPO_4. The pH of the medium was adjusted after sterilization by the method and apparatus described by CHRISTENSEN and FULMER (**1**).

Experimental method

The incubator in which the organisms were grown was placed in a room which for several years had been used for water analysis and hence was unusually free from contamination with ammonia or nitric acid fumes. A current of air passed through potassium permanganate, sodium hydroxide, and sulphuric acid solutions was passed through the incubator.

Each analysis was run on a separate flask, several hundred of which were required for the experiments described. All flasks including the blanks were inoculated to a count of one (250,000 cells per cc.), a blank being used for each pH value. The flasks designed for blanks were sterilized with live steam in order to kill the yeast.

The fact that nitrogenous compounds were not taken from the air in a period of 6–8 weeks through the pH range used is shown by the data in table III.

TABLE III

NITROGEN CONTENT OF BLANKS (MG./100 CC.)

EXPERIMENT	A	B	C						
TIME (WEEKS)	6	6	1		2		4		6
METHOD	I	I	I	II	I	II	I	II	I
pH									
4.0	12.70	12.51	29.48	39.19	30.94	42.74	29.41	40.99	....
5.0	12.60	13.18	29.41	42.09	30.30	41.81	29.83	41.66	29.41
6.0	12.80	12.20	29.41	40.39	31.25	42.37	30.12	41.32	....
7.0	....	12.20	29.24	39.56	30.30	41.81	30.30	41.32	29.76
8.0	12.70	12.82	29.59	40.42	30.12	41.32	30.30	40.42	29.94
9.0	12.70	12.20	29.41	42.09	29.83	....	30.49	41.53	29.76
Average	12.70	12.53	29.42	40.62	30.46	42.01	30.09	41.21	29.72

Mean I = 30.08; II = 41.25.

Experiments A and B were run with molasses containing 0.20 per cent. N (Kjeldahl) and C with molasses containing 0.41 per cent. N (Kjeldahl).

I and II refer to the Kjeldahl and modified Kjeldahl respectively.

Two methods of analysis were used: I. The regular Kjeldahl method. II. The modified method. This involves the addition of hydrogen peroxide to 15 per cent. and 0.1 per cent. sulphuric acid as previously described by CHRISTENSEN and FULMER (**2**). The mixture was evaporated nearly to dryness over a slow flame. After the residue was cooled the regular Kjeldahl procedure was employed.

The ammonia was determined by Nesslerization.

Experiments with *Mycoderma*

The results of the experiments with *Mycoderma* are given in table IV. The values for the blanks have been previously given in table III.

TABLE IV

ANALYSIS OF NITROGEN IN CULTURES OF *Mycoderma* (MG./100 CC.)

EXPERIMENT	A						B*				
TIME (WEEKS)	1	2	3	4	6	8	1	2	3	6	8
METHOD	I	I	I	I	I	I	I	I	I	I	I
pH											
4.00						- 0.9					- 0.8
4.50						- 1.5					+ 0.4
5.00					- 4.5	- 2.1					+ 0.6
5.25					......	......					......
5.50					- 1.9	- 1.2					+ 0.9
5.75					......	......					......
6.00					......	- 0.5					+ 0.5
6.20					- 0.5	......					......
6.25					......	......					......
6.40					- 1.3	......					......
6.50					......	- 2.1					+ 0.2
6.75					......	......					......
6.80					- 3.2	......					......
7.00					- 2.5	- 2.1					- 1.1
7.20	- 3.6	- 3.4	- 4.7	- 4.2	- 1.9	- 0.9	- 5.7	- 4.2	- 4.0	+ 2.6	+ 0.4
7.25	......	......	......	......	......	......	......	......	......	......	......
7.40	- 2.6	- 3.5	- 2.6	- 2.5	+ 1.2	- 0.7	- 5.1	- 2.9	- 2.7	+ 1.2	+ 0.2
7.50	......	......	......	......	......	......	......	......	......	......	......
7.60	- 3.2	- 4.5	- 3.6	- 3.4	+ 2.0	+ 1.3	- 4.7	- 3.8	- 4.6	......	±0.0
7.75	......	......	......	......	......	......	......	......	......	......	......
7.80	- 1.0	- 1.2	- 5.3	- 3.6	+ 2.3	+ 3.8	- 4.4	- 6.9	- 4.5	+ 0.2	+ 0.2
8.00	- 0.9	- 3.2	- 7.0	- 3.7	+ 1.6	+ 4.9	- 3.5	- 6.1	- 4.1	+ 0.2	+ 0.4
8.25					......	......					......
8.50					- 0.8	+ 3.3					+ 0.2
9.00					- 1.8	+ 2.4					- 1.7
9.50						+ 2.0					- 1.0

pH and pH′ represent the hydrogen-ion concentration before inoculation, and after growth had taken place, respectively.

* This medium contained 10 g. sucrose per 100 cc. in addition to the molasses.

It will be noted that the results in experiment C are more erratic than those in experiment A. The latter experiment was run on a molasses con-

TABLE IV—(*Continued*)

ANALYSIS OF NITROGEN IN CULTURES OF *Mycoderma* (MG./100 CC.)

EXPERIMENT	C										
TIME (WEEKS)	1				2				4		6
METHOD			I	II			I	II	I	II	I
pH	pH′	Count/10	I	II	pH′	Count/10					
4.00	5.02	2.6	-1.0	-3.0	5.36	10.8	-0.3	+3.3	+3.2	-2.2	-5.1
4.50	5.71	2.2	-1.9	-3.1	6.11	9.5	-0.8	+9.0	+1.1	+1.1	-1.4
5.00	6.38	1.3	-3.5	......	6.34	10.2	+0.9	+12.2	±0.0	+2.3	......
5.25	6.05	2.2	-0.6	-2.8	6.39	12.3	+3.3	+2.0	+1.2	+3.5	+0.4
5.50	6.00	2.9	+0.7	-2.1	6.34	11.0	+1.3	+0.7	+1.6	+1.3	-3.2
5.75	6.28	2.5	+1.0	+6.9	6.54	13.5	+1.1	+6.6	+2.4	+3.4	......
6.00	6.30	2.9	-0.3	+7.0	6.52	10.4	......	+6.6	+0.7	+3.5	......
6.20	......	......	......	......	......	......	......	......	......	......	......
6.25	6.85	1.3	-0.7	+8.7	7.03	9.4	+3.1	+6.1	+2.0	+0.5	......
6.40	......	......	......	......	......	......	......	......	......	......	......
6.50	......	......	+0.1	+5.5	7.27	8.8	+1.0	+8.7	-0.6	-0.3	-2.3
6.75	7.22	1.9	......	......	7.69	5.8	+1.3	+0.6	+0.4	-1.2	-0.5
6.80	......	......	......	......	......	......	......	......	......	......	......
7.00	7.31	1.9	-0.5	+4.3	7.69	7.3	-1.5	+9.3	+1.0	+0.1	-4.2
7.20	......	......	......	......	......	......	......	......	......	......	......
7.25	7.35	1.2	±0.0	-7.5	7.75	8.2	-1.4	+1.5	+2.2	+1.5	......
7.40	......	......	......	......	......	......	......	......	......	......	......
7.50	7.48	1.8	-0.5	-9.0	8.10	7.7	-1.4	......	......	+0.4	-2.6
7.60	......	......	......	......	......	......	......	......	......	......	......
7.75	7.67	1.9	-3.5	-2.7	8.12	7.3	+0.3	+6.4	......	+3.06	......
7.80	......	......	......	......	......	......	......	......	......	......	......
8.00	7.77	1.5	......	-2.3	8.31	8.7	+1.2	+1.1	-0.6	-2.8	-2.4
8.25	7.83	1.0	-3.4	-1.2	8.37	8.6	+3.3	+1.0	+0.3	-1.0	-3.5
8.50	7.91	......	-2.4	-1.2	8.55	8.2	-1.1	+5.0	+1.6	+2.7	+1.6
9.00	8.54	......	-1.0		8.74	6.1	-2.3	+6.1	+1.6	......	
9.50											

taining about one-half the nitrogen in the former. This may account in part for the irregular results. The larger the amount of nitrogen present the greater is the probability of error in dealing with small differences. From the data certain conclusions may be drawn.

1. The amount of nitrogen in the culture is a function of pH and time.

2. The modified Kjeldahl method shows much greater gains as well as greater losses than the regular Kjeldahl method. In our previous communication we had suggested that the fact that the pH in which there was the greatest preliminary loss in nitrogen coincided with the final greatest

gain, was due to analytical method. Evidently the new method does not solve the difficulty.

3. Whether a gain or loss of nitrogen is found depends upon the method used for analysis. Conditions determined to be optimal by one method of analysis may not appear optimal when another analytical method is used.

4. After a long period the cultures all begin to lose nitrogen as determined by both methods, the loss being relatively greater with the modified than with the Kjeldahl method.

5. There is no obvious correlation between the number of cells and the phenomena discussed above.

6. The pH of the medium changes in such a way that the alkaline become more acid and the acid more alkaline narrowing the pH range of the series from a range of about 5.5 units initially to about 3.5 in one week and to 3.4 in two weeks.

Experiments with *Saccharomyces cerevisiae*

The results for *Saccharomyces cerevisiae* are given in table V.

The results are erratic due to the small changes involved, but the general tendencies are the same as those for *Mycoderma*. The modified method

TABLE V

ANALYSIS OF NITROGEN IN CULTURES OF *Saccharomyces cerevisiae* (MG./100 CC.)

TIME (WEEKS)	1				2				4		6
pH	pH′	Count	I	II	pH′	Count	I	II	I	II	I
4.00	4.00	310	−0.33	−5.6	4.45	294	−2.1	−0.3	+0.7	−0.9	......
4.50	4.60	380	−0.49	−5.9	4.68	303	−1.4	−1.6	−0.3	+4.0	−3.1
5.00	5.00	345	−0.33	−6.1	4.85	304	+2.5	−2.5	+0.3	+1.5	−3.8
5.25		325	−0.49	−5.5	4.96	304	+0.2	−2.8	−1.3	+4.9	+0.8
5.50		360	−0.33	−5.8	5.06	348	+1.9	......	+1.5	+4.1	−2.70
5.75		355	−0.49	−6.1	5.15	348	+0.0	......	+0.4	+0.4	−1.5
6.00	5.20	415	−1.3	−5.8	5.74	348	−3.3	−2.8	−2.7	+1.2	......
6.25		390	−1.3	−6.1	5.80	386	+0.4	......	+0.2	+1.8	−1.5
6.50		400	−1.3	−5.3	6.08	382	+1.2	......	+1.5	+5.5	......
6.75		370	−1.5	−5.5	6.11	408	−1.2	......	......	−3.3	......
7.00	6.40	334	−2.2	−5.5	6.14	394	−0.7	−1.3	+2.1	−4.4	......
7.25		360	−2.6	−6.4	6.20	376	−0.3	−0.8	+1.1	+1.3	−1.5
7.50		370	−3.1	−6.1	......	......	−0.7	−1.3	−0.5	−3.3	......
7.75		445	−2.6	−6.5	......	......	−2.1	−1.6	+0.5	......	......
8.00		......	−2.3	−6.1	6.24	372	−1.3	......	+0.0	+0.5	−5.9
8.25	6.60	350	......	−5.5	6.28	393	−0.7	−0.8	+1.2	−0.3	−6.0
8.50		360	−2.3	−5.5	6.43	342	−1.2	......	+2.0	+1.1	−0.2
9.00	6.80	285	−0.86	−5.5	6.62	301	+0.8	......	+0.2	+0.5	......

again shows that the greatest losses and the greatest gains are both functions of pH. There is the same preliminary loss followed by gain and with a subsequent loss after six weeks. The striking difference is in the pH change which is much greater with the *Saccharomyces*. An original range of 5 pH units is in two weeks narrowed down to 2.2 units. This means that the pH range finally involved is less than for the *Mycoderma*. There is no obvious correlation between the number of cells and the losses or gains in nitrogen. Through the pH range tested the changes in nitrogen content are much less than with the *Mycoderma*; however, as indicated above, the pH range is finally less than with the *Mycoderma*.

Summary

The nitrogen content of cultures of *Mycoderma* and of *Saccharomyces cerevisiae* in molasses at various values of pH has been followed by two methods of analysis, the regular Kjeldahl and a modified Kjeldahl previously described from this laboratory. The modified method magnifies both the losses and gains in nitrogen. The maximum losses and maximum gains are not in all cases at the same pH by both methods. Following the maximum gain there is a loss in nitrogen. The nitrogen content of the cultures is dependent then upon pH, time and method of analysis. The results indicate that neither method gives all the nitrogen at any given pH or time interval. It is evident that results on nitrogen fixation are more likely to err on the negative than on the positive, that is, there probably is more fixation than any available method indicates.

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THE DEVELOPMENT OF CHLOROPHYLL IN SEEDLINGS IN DIFFERENT RANGES OF WAVE LENGTHS OF LIGHT*

J. D. SAYRE

(WITH THREE FIGURES)

Introduction

While investigating the behavior of stomata in different kinds of light it was noticed that no chlorophyll developed in seedlings grown under some of the colored glass plates used in that work. The object of this investigation was to find out more fully, if possible, the wave lengths of light necessary for the development of chlorophyll in plants. A search of the literature yielded very little information on the subject. The only record of any other work of this kind is in PALLADIN's Plant Physiology (**4**), where WIESNER used double walled bell jars with different colored solutions to separate the spectrum into different parts. He reported that chlorophyll was formed in both the red-orange-yellow region, produced by potassium dichromate, and also in the green-blue-violet region produced by copper sulphate. The non-luminous heat rays were not effective. There is plenty of information on the effects of various wave lengths of light on photosynthesis, especially on those wave lengths which produce maximum photosynthesis. This is very well summarized by SPOEHR (**6**).

Methods used

Seedlings were grown under colored glass plates and their color noted. No measurements of the amount of chlorophyll in the tissue were made. The relative greenness of the plants when compared with controls grown under all wave lengths of light was taken as a measure of the chlorophyll present. All plants were grown under similar temperature, humidity, and soil conditions, the variable being the different wave length of light. Seedlings of corn, wheat, oats, sunflower, radish, mustard, and bean were used in this work.

The colored glass plates used were the 6½ inch square ray filters manufactured by the Corning Glass Works, Corning, N. Y. The same series as used in the spectral glass houses of the Boyce Thompson Institute, reported by POPP (**5**), were employed with a few additions. The manufacturers'

* Paper from the Department of Botany, The Ohio State University, no. 210.

numbers of these plates are G86B, G38L, G38H, G34, G24, G124J, G584J, G55A, G585L, and G586A.

The transmission data for these glass plates were obtained from the Bureau of Standards Technologic Papers by COBLENTZ and EMERSON (1), GIBSON and MCNICHOLAS (2), and GIBSON, TYNDALL, and MCNICHOLAS (3). These data were converted by means of the chart and instructions given by GIBSON and MCNICHOLAS (2), values for the average thickness of the plates used. The values for combinations of two or more plates is the product of the data for each plate separately. All these data are given for an equal energy spectrum. The percentage of total transmission for each plate or each combination of plates was obtained by comparing the areas under the transmission curves when they were plotted on cross-section paper. These data were calculated for a spectrum extending from 300 mμ to 770 mμ, 770 mμ being the limit of visible spectrum as given by GIBSON and MCNICHOLAS (2). The transmission curves for these glass plates are shown in fig. 1 and fig 2. These data can be used only for plates of the thickness indicated in each case. The limits of transmission for each plate were checked by means of a wave length spectrometer loaned by the Physical Chemistry Department of the Ohio State University.

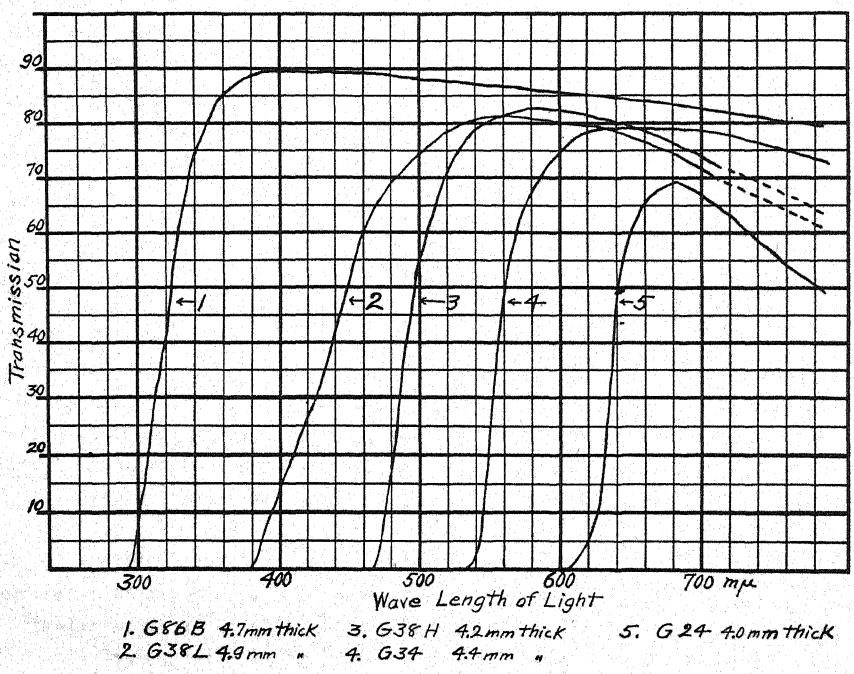

FIG. 1. Transmission curves of glass plates used in the experiments.

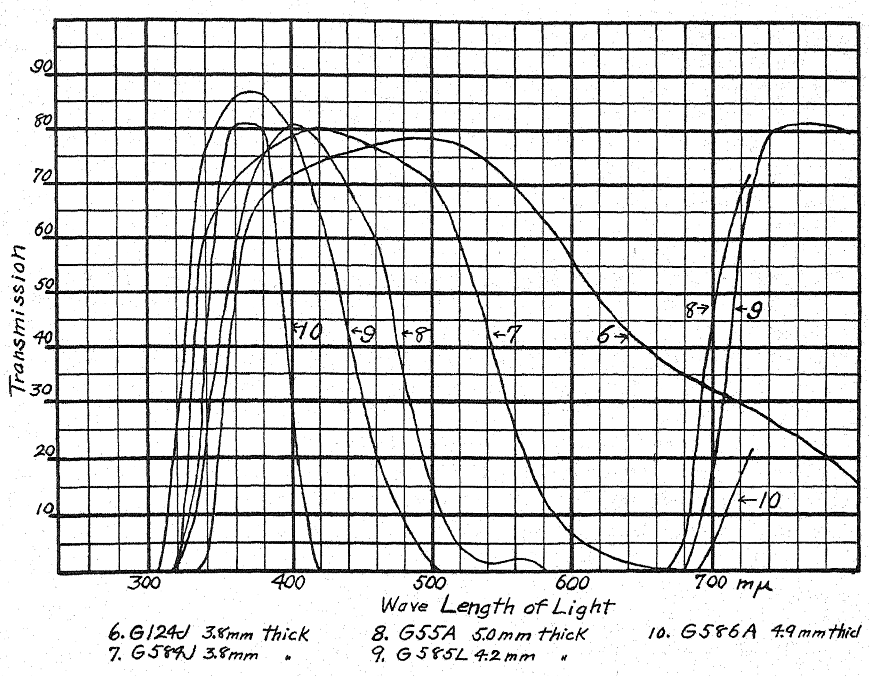

FIG. 2. Transmission curves of glass plates used in the experiments.

The data for the relative spectral distribution of radiant energy from the 400 watt Mazda C light were taken from GIBSON and MCNICHOLAS (2). The percentage of energy from the Mazda C light transmitted by each plate was found by calculation. The values of relative spectral distribution of energy from the Mazda C light were multiplied by the transmission value at each wave length and curves of these values plotted on paper. The areas under each curve were compared and reduced to percentages. These percentages are comparable when all plants are placed at equal distances from the light.

The plants were grown under ventilated, light-proof tin boxes which held four glass plates. The boxes were painted white (MgO paint) inside, and with aluminum paint on the outside. The seeds were planted directly in the soil when the experiments were conducted out of doors, and the tin boxes were sunk several inches in the soil to make a light-tight seal. In the greenhouse, flats of soil were used and the tin boxes were sunk for several inches into a large tray of soil. For the experiments under artificial light a large box was used. The plants were placed one meter from this light. Many experiments were tried, and many calculations of data were necessary for this work which has covered a period of several years at interrupted

intervals. The results of only two typical experiments are given since similar results were obtained each time.

Results

Table I gives the results of a series of experiments carried out in the greenhouse in the fall and winter of 1926–27. This series covered a period of six to seven weeks, since only five sets of plates and a control could be used at one time. The numbers of the glass plates are given with their limits of transmission, and the percentage of an equal energy spectrum transmitted by each. The development of chlorophyll is expressed by

TABLE I

TRANSMISSION DATA AND DEVELOPMENT OF CHLOROPHYLL

COMBINATIONS OF GLASS PLATES	LIMITS OF TRANSMISSION	TRANSMISSION OF AN EQUAL ENERGY SPECTRUM	DEVELOPMENT OF CHLOROPHYLL
	mμ	Per cent.	
G86B	296 – infra red	70.0	Green (control)
G38L	380 – " "	54.0	" as control
G38H	464 – " "	44.5	" " "
G34	528 – " "	33.5	" " "
G24	601 – " "	18.3	" " "
G24 + G55A	670 – " "	6.6	No trace of green*
G24 + G586A	680 – " "	5.7	" " " "
G24 + G585L	690 – " "	2.1	" " " "
G24 + G584J	601 – 680	Less than 0.1	Trace of green
G34 + G584J	528 – 680	1.0	Almost as green as control
G38H + G584J	464 – 680	6.7	" " " " "
G38L + G584J	380 – 680	14.4	Green as control
G34 + G55A	528 – 580	Less than 0.1	No trace of green
G38H + G55A	464 – 580	0.5	Trace of green
G38L + G55A	380 – 580	5.7	Almost as green as control
G38H + G585L	464 – 506	0.2	No trace of green
G38L + G585L	380 – 506	2.6	Almost as green as control
G38L + G586A	380 – 420	0.2	No trace of green
G586A	320 – 420	10.7	Trace of green
G585L	300 – 506	19.3	Almost as green as control
G55A	325 – 580	20.1	" " " " "
G584J	300 – 680	34.8	Green as control

* Faint trace of green in sunflower seedlings in one experiment

different amounts of the green color when compared with plants grown in full daylight. The plants which showed no trace of green were yellow in color similar to etiolated seedlings. These data seem to show two facts:

first, that there is no development of chlorophyll in radiant energy of wave lengths longer than 680 mμ; and second, that all wave lengths of the remaining visible and ultra-violet spectrum are effective if the intensity is high enough. Traces of green color were detected in all combinations where wave lengths shorter than 670 mμ were transmitted except in those where the percentage of total energy transmitted was very low. It must be remembered that the plants were grown in daylight and were not exposed to an equal energy spectrum, so that the transmission percentages are not strictly comparable. It was to get around this difficulty that the series under artificial light was conducted. Here the intensity is constant and the relative spectral distribution and total percentage transmitted can be calculated.

Figure 3 shows the relative spectral distribution of energy under the different combinations of plates used with artificial light. The relative

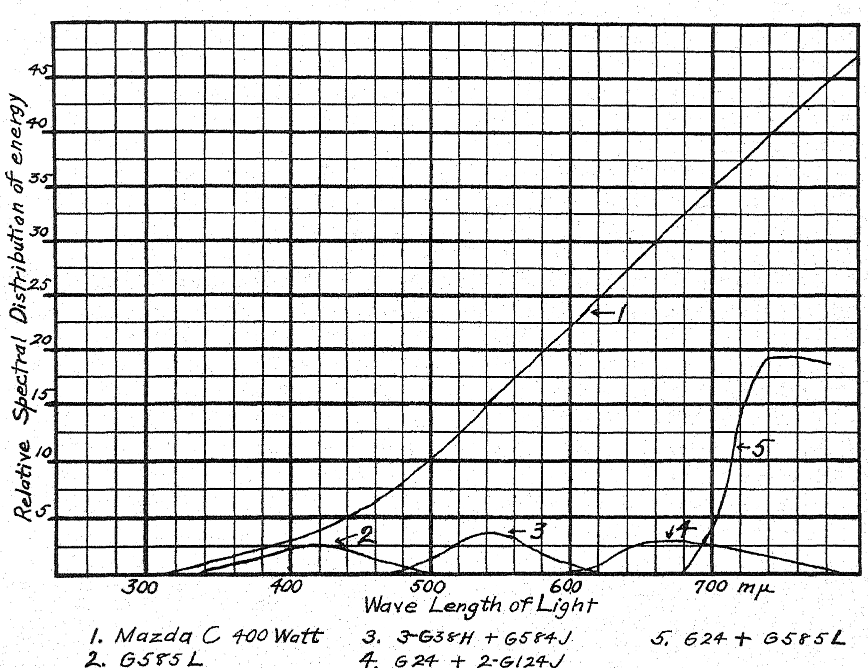

FIG. 3. Relative spectral distribution of energy under different combinations of glasses used.

spectral distribution of energy from the Mazda C 400 watt lamp is also given. The combination 3-G38H + G584J and 624 + 2-G124J were chosen to give values as near as possible to those for G585L. The plants under these plates were all the same distance from the light.

Table II gives the results of an experiment under artificial light. The combinations of plates used, their limits of transmission, and the relative percentage of the total energy from the Mazda C transmitted by each are given. The seeds were germinated and the seedlings grown in the dark to a height of several inches before the light was supplied. The time required for the development of the green color under the different lights is given.

TABLE II

TRANSMISSION DATA AND DEVELOPMENT OF CHLOROPHYLL

Combinations of glass plates	G585L	3–G38H + 584J	G24 + 2–G124J	G24 + G585L
Limits of transmission	320–506 mμ	464–630 mμ	600–800 mμ	680–infra red
Percentage of energy from 400 watt Madza C transmitted	3.3	3.1	4.0	18.0
Time	Relative development of chlorophyll in the seedlings			
Feb. 10, '27	Yellow	Yellow	Yellow	Yellow
9:30 A. M.	Yellow	Yellow	Yellow	Yellow
10:15 A. M.	Yellow	Yellow	Yellow	Yellow
11:15 A. M.	Yellow	Yellow	Yellow	Yellow
12 Noon	Yellow	Yellow	Faint trace green	Yellow
1:00 P. M.	Yellow	Faint trace green	Trace green	Yellow
2:00 P. M.	Trace green	Trace green	Green	Yellow
3:00 P. M.	Trace green	Trace green	Green	Yellow
4:00 P. M.	Trace green	Green	Green	Yellow
Feb. 11, '27				
10:00 A. M.	Trace green	Green	Green	Yellow

These data show, as in the other experiments, that even after 24 hours there is not the faintest trace of green in light of wave lengths longer than 680 mμ. The relative intensity of this radiant energy is three times that of the other parts of the spectrum in which chlorophyll development is very pronounced. The data also show that, for approximately equal energy values in the remaining visible and ultra-violet spectrum, there is a difference in their effect on chlorophyll development. This effect seems to be that the red rays are more effective than the green, and the green more than the blue. When the effective energy under the G24 + 2-G124J combination is considered to extend only to 680 mμ, since longer wave lengths

of radiant energy have no effect, the relative transmission percentage becomes 2.5 per cent. instead of 4.0 per cent. as compared with 3.1 per cent. in the green and 3.3 per cent. in the red. The relative effectiveness of the red region is still more apparent.

The fact that there is no development of chlorophyll beyond 680 mμ is very striking because the center of the band of maximum absorption by chlorophyll and the band of greatest efficiency of radiant energy in photosynthesis are at about 675 mμ (SPOEHR). It seems, therefore, that the effectiveness of radiant energy in chlorophyll development increases with the wave length up to about 680 mμ and then ceases abruptly.

Conclusions

1. Wave lengths of radiant energy longer than 680 mμ are not effective in the formation of chlorophyll in seedlings of corn, wheat, oats, barley, beans, sunflowers, and radish.
2. All other regions of the remaining visible and ultra-violet spectrum (to 300 mμ) are effective provided the energy value is sufficient.
3. For approximately equal energy values in these regions, the red rays are more effective than the green, and the green more than the blue.
4. The effectiveness of radiant energy appears to increase with wave length to about 680 mμ and then to end abruptly.

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SIMILARITY BETWEEN PHYSICOCHEMICAL AND BIOLOGICAL REACTIONS*

C. P. SIDERIS

Students of physiological mycology have long recognized that different microorganisms behave differently both in vigor of growth and in the character of their biochemical reactions when grown on chemically identical substrata. Some of these phenomena have been interpreted rather satisfactorily by certain physicochemical laws, others, however, have either not been explained at all or attributed to the operation of certain vitalistic forces.

Every student of life behavior realizes the difficulties involved in resolving biological phenomena into simple physicochemical reactions. There are occasional cases where one is able to apply certain physicochemical laws. Such a case is presented in the following pages.

There are two proteins with distinct physicochemical properties in the stem of the pineapple plant: Protein-A which is isoelectric at pH 6.4, and protein-B at pH 4.8. Qualitative chemical tests varied in the two proteins as follows:

Protein-A: Biuret (+++), Xanthroproteic (++),
Sulphur (–), Molisch (–).
Protein-B: Biuret (+), Xanthroproteic (+),
Sulphur (+), Molisch (–).

The separation and treatment of these proteins is discussed in another paper, which will be published elsewhere.

Protein-A has been used for the present study. This protein after a thorough purification by means of dialysis was further diluted with distilled water. This solution was now thoroughly stirred by a mechanical stirrer in order to bring about uniform distribution of the protein particles, and distributed to a number of Erlenmeyer flasks, each one receiving 100 cc. drawn off by means of a pipette. To each one of these flasks different volumes of either 0.1 N. HNO_3 or NaOH were added accordingly to table I.

The table indicates that the isoelectric point of protein-A of the pineapple stem is close to pH 6.43. It, also, shows that this protein may react at pH values below the isoelectric point to form salts, such as protein-

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TABLE I

BEHAVIOR OF PROTEIN-A WITH ACID AND ALKALI ADDITIONS

SAMPLE NO.	REAGENT ADDED		PH	TURBIDITY	BEHAVIOR WITH SALTS		REMARKS
	HNO_3	NaOH			$K_4Fe(CN)_6$	$AgNO_3$	
	cc.	cc.					
1	4.0		2.63	–	+++	–	
2	3.5		2.67	–	+++	–	
3	3.0		2.70	–	+++	–	
4	2.5		2.88	–	+++	–	
5	2.0		3.00	+	+++	–	
6	1.5		3.13	+	+++	–	
7	1.0		3.33	+	++	–	
8	0.5		4.16	++	++	–	
9	0.25		5.32	+++	+	–	
10	0.125		6.43	++++	–	–	(Isoelectric point)
11	0	0	7.30	++	–	+	
12		0.125	8.65	+	–	++	
13		0.25	9.45	–	–	+++	
14		0.5	9.90	–	–	+++	
15		1.0	10.42	–	–	+++	
16		1.5	10.58	–	–	+++	
17		2.0	11.05	–	–	+++	

ferrocyanate, where the protein takes the place of the cation, and at values above the isoelectric point to form salts, such as silver-proteinate, where the protein takes the place of the anion. HARDY was the first to point out, a number of years ago, that proteins may act as ampholytes. Further investigations and confirmation of this and other properties of proteins came in later years through the researches of PAULI, RONA, MICHAELIS, SØRENSEN, LOEB and others.

The two reactive radicals of a protein that make it amphoteric are the amino (NH_2) and carboxyl (COOH) groups. The former is capable of combining with anions and the latter with cations, to form salts.

Growth behavior of fungi on pineapple protein–A

The different solutions of protein-A as reported in table I were divided into 25-cc. portions and used for the growth of the following organisms:

Fusarium martii,
Verticillium sp.,
Penicillium sp.

Both mycelium and spores were used for the inoculation of the different cultures, the latter being used wherever possible. The cultures were kept at room temperature, that is at about 27° C., and compared with cultures of the same organisms grown on solid and liquid nutrient media. The growth of the different organisms is recorded in table II.

TABLE II

GROWTH OF ORGANISMS ON PROTEIN-A

SAMPLE NO.	pH	GROWTH BEHAVIOR OF		
		Fusarium	*Verticillium*	*Penicillium*
1	2.63	−	+	++
2	2.67	−	+	++
3	2.70	−	++	++
4	2.88	−	++	++
5	3.00	−	++	+++
6	3.13	−	++	+++
7	3.33	−	+++	+++
8	4.16	−	++	++
9	5.32	−	++	++
10	6.43	−	−	−
11	7.30	+	−	+
12	8.65	++	−	+++
13	9.45	+++	−	+++
14	9.90	+++	−	++
15	10.42	++	−	++
16	10.58	++	−	++
17	11.05	+	−	+

Discussion

Protein as such is not available for the use of a fungus. We know that organisms release enzymes to decompose and make available not readily available substances. Very little is known of the operative mechanism of enzymes, because there is no satisfactory evidence of their chemical constitution. Certain investigators have stated at different times that enzymes that decompose proteins are proteins and those that decompose carbohydrates are carbohydrates. Just how true this statement is the writer is not in a position to say. If we take it for granted, however, that the case may be such and attempt to interpret the results obtained with the different organisms accordingly, we have the following condition.

Assuming, *a priori*, that the enzyme released by each of the three different organisms and causing the breaking down of protein-A is of protein composition, we may be justified then in attributing its chemical reactivity

to either one of its two active component radicals, namely, NH_2 or COOH. With the above assumption in mind one may easily visualize the reactions taking place between the enzymes of *Fusarium* and *Verticillium*, on the one hand, and the pineapple protein, on the other, but it is considerably more difficult to understand the operative mechanism of *Penicillium*.

In order to make some of the points more clear let us consider the types of chemical reactions that are possible in each case.

It is possible that the condition of the protein at the isoelectric point, and above or below this point, is as follows:

Isoelectric point, $HOOC—R—NH_2$
Below isoelectric point, $HOOC—R—NH_2 + H^+ \rightarrow (HOOC—R—NH_3)^+$
Above isoelectric point, $HOOC—R—NH_2 + OH^- \rightarrow (OOC—R—NH_2)^- + H_2O$

The very same types of conditions may be ascribed to the enzymes of the different organisms according to our assumption that they are of protein composition. As the isoelectric point of the different enzymes is not known it is difficult accurately to know their reactivity at different pH values. The results already obtained indicate that the enzyme of *Fusarium* operated only at pH values above the isoelectric point of the pineapple protein and that of *Verticillium* at values below the isoelectric point. This sort of behavior reveals the presence of two enzymes with opposite electrical charges, that of *Fusarium* carrying a positive and that of *Verticillium* a negative charge.

One wonders whether the enzymes of *Fusarium* and *Verticillium* are of proteinaceous composition or not. They do not seem to be amphoteric but are charged either positively or negatively through a wide range of H-ion concentrations, judging from their reactivity with the pineapple protein. Exactly what conditions prevail with the enzyme of *Penicillium*, which shows amphoteric behavior, is difficult to explain. From the evidence presented so far, it is shown that the latter enzyme must be affected somewhat differently than the pineapple protein by hydrogen ions, and it assumes an electric charge opposite to that of the protein. In accordance with our former assumption it is possible to have the following conditions with the different enzymes:

For *Fusarium*:

At the isoelectric point

$$(HOOC\text{–protein–}NH_2) + (HOOC\text{–enzyme–}NH_3)^+ = \text{no reaction.}$$

Above the isoelectric point

$$(OOC\text{–protein–}NH_2)^- + (HOOC\text{–enzyme–}NH_3)^+ \longrightarrow HOOC\text{–protein–}NH_2 + HOOC\text{–enzyme–}NH_2 = \text{reaction.}$$

Below the isoelectric point

$(HOOC\text{–protein–}NH_3)^+ + (HOOC\text{–enzyme–}NH_3)^+ = \text{no reaction.}$

For *Verticillium:*

At the isoelectric point

$HOOC\text{–protein–}NH_2 + (OOC\text{–enzyme–}NH_2)^- = \text{no reaction.}$

Above the isoelectric point

$(OOC\text{–protein–}NH_2)^- + (OOC\text{–enzyme–}NH_2)^- = \text{no reaction.}$

Below the isoelectric point

$(HOOC\text{–protein–}NH_3)^+ + (OOC\text{–enzyme–}NH_2)^- \longrightarrow HOOC\text{–protein–}NH_2 + HOOC\text{–enzyme–}NH_2 = \text{reaction.}$

It is questionable in the case of *Penicillium* whether there is only a single enzyme operating throughout such a wide range, namely, pH 2.0 to 11.00. There might be two enzymes, one carrying a negative electric charge and operating below the isoelectric point of the protein and the other with a positive electric charge and operating above the isoelectric point. Whether similar conditions are to be found with other proteins and fungi remains to be seen and further studies are in progress.

Summary

An attempt is made in this study to compare biological with physicochemical reactions and to interpret the former in terms of the latter. To just what extent this is feasible remains to be proved by further experimentation.

It has been found that *Fusarium martii, Verticillium* sp. and *Penicillium* sp. do not grow in isoelectric pineapple stem protein-A. *Fusarium* may grow in solutions of this protein having a pH value above that of the isoelectric point of the protein. *Verticillium* behaves exactly reverse, that is, it grows only in solutions of the protein having pH values below that of the isoelectric point. *Penicillium* sp. was found to grow in solutions of the protein having a pH value either above or below the isoelectric point.

The writer is indebted to Dr. A. L. DEAN for many helpful suggestions, and to Miss B. H. KRAUSS for technical assistance.

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PHOTOSYNTHESIS IN ABSENCE OF OXYGEN*

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BEIJERINCK (1) was the first to use luminous bacteria as detectors of oxygen in the study of photosynthesis. He found that extracts of destroyed cells containing chloroplasts, mixed with luminous bacteria whose luminescence had disappeared because of lack of oxygen, would, when illuminated, produce photosynthetic oxygen that was detected by the luminescence of the bacteria.

MOLISCH (5, 6) later found that leaves carefully dried, powdered, and suspended in emulsions of luminous bacteria could also photosynthesize when illuminated.

The simplicity and sensitivity of this method of study make it especially valuable for exploratory studies on photosynthesis under different conditions and especially for studies on photosynthesis in absence of oxygen, since the luminous bacteria themselves may act as the means of absorbing oxygen.

Statements in the literature as to the necessity of oxygen for photosynthesis are somewhat conflicting, but the general conclusion seems to be that oxygen is necessary (7, 8), different plants differing in their resistance to lack of oxygen. WILLSTÄTTER and STOLL (10) found *Pelargonium* to be quite sensitive, while *Cyclamen* was very resistant to lack of oxygen.

Despite the statements as to the necessity of oxygen for photosynthesis there is no theoretical reason why the first step in photosynthesis, the splitting of CO_2, which is the step detected by luminous bacteria, should fail in absence of oxygen. It would seem that further experiments in which it is certain that the oxygen is completely removed are desirable.

In many experiments where gases are passed over cells to remove the oxygen, the gases may contain traces of oxygen which are easily detected by the luminescence of luminous bacteria. HARVEY and MORRISON (3) have found that light can just be detected in an emulsion of luminous bacteria 3 cm. thick when in equilibrium with 0.0053 mm. O_2 or 0.0007 volume per cent. of oxygen. This will give some idea of the sensitivity of luminous bacteria as oxygen detectors. However, an emulsion of luminous bacteria becomes completely dark if undisturbed, and can reduce many dyes whose oxidation-reduction potential is well known. A knowledge of the potential of the dye allows us to calculate the pressure of oxygen with which equimolecular quantities of reduced and oxidized dye would be in equilibrium.

* From the Marine Biological Laboratory, Woods Hole, Massachusetts.

The particular strain and concentration of bacteria used in these experiments reduced methylene blue in 3–4 minutes and indigo disulphonate in about an hour. Indigo monosulphonate was not reduced.

The potential of an equimolecular mixture of reduced and oxidized methylene blue is about +0.02 volts at pH = 7 which corresponds to an oxygen pressure of about 10^{-53} atmospheres, a figure that has no physical significance. We may at least say that the last molecule of oxygen is removed by luminous bacteria and that they produce perfect anaerobic conditions.

As luminous bacteria are marine forms and live well when emulsified in sea water, I have added various kinds of marine algae to tubes containing emulsions of luminous bacteria which had become dark through using of oxygen. After remaining in the oxygen-free sea water for a given time the algae were illuminated with a 60 watt straight filament Mazda lamp at a distance of 12 cm. for a few seconds.

Not only do the bacteria use oxygen but they also produce CO_2, changing the pH of the sea water from 8 to 6.9–7 in the course of an hour. A pH of 7 corresponds to 4 mm. CO_2, according to HENDERSON'S and COHN'S (4) sea water pH–CO_2 pressure curve. On blowing air through the sea water containing the bacteria its pH returns to about 8 showing that non-volatile acids are not produced in large amount. The bacteria may then be regarded as ideal means of removing oxygen since they at the same time add the CO_2 necessary for photosynthesis.

The actual results are given in table I. The algae were recently collected, and kindly identified for me by Dr. W. R. TAYLOR. The number of + signs indicates roughly the intensity of the luminescence, and the − signs mean no luminescence.

It will be observed that nearly all the algae readily form oxygen under the conditions of the experiment. Since *Grinellia* gave no luminescence I concluded that the specimen was either dead or in poor condition. This experiment will, therefore, serve as a control to show that luminous bacteria themselves produce no oxygen or luminescence when illuminated. It should be mentioned that they do luminesce when suspended in sea water after exposure in a quartz tube to light from a quartz Hg-vapor lamp. One might suppose that this experiment indicated a splitting of the CO_2 of sea water by ultra-violet light, but it is perhaps better interpreted as a production by the ultra-violet light, of H_2O_2, which then decomposes with liberation of oxygen. TIAN (9) found H_2O_2 to be formed in absence of oxygen when sea water was exposed to ultra-violet. The convenience of luminous bacteria as oxygen detectors suggests their value in experiments on photosynthesis *in vitro* upon which I am at present engaged.

TABLE I

PHOTOSYNTHESIS OF MARINE ALGAE

PLANT	OXYGEN PRODUCTION ON ILLUMINATION AFTER OXYGEN-FREE CONDITIONS FOR				
	1 minute		15 minutes	30 minutes more	
	Illuminated		Illuminated	Illuminated	
	3 sec.	30 sec.	3 sec.	3 sec.	30 sec.
Green algae					
Cladophora gracilis	+	+		+	
Bryopsis plumosa	+	+		+	
Enteromorpha intestinalis	+	++		+	
Ulva lactuca rigida	+	+	+	+	
Chaetomorpha linum	++	++	++	++	
Red algae					
Polysiphonia variegata	+	–		+	–
Polysiphonia violacea	+	++	+	+	
Polysiphonia nigrescens	+			+ faint	
Chondrus crispus	+	+	+	+	
Lomentaria uncinata	+	+	+	+	
Corallina officinalis	+	+	+	+	
Grinellia americana	–	–	–	–	–
Brown algae					
Fucus vesiculosus	++	+++	++	++	
Ascophyllum nodosum	++	+++	++	++	
Ectocarpus siliculosum	+ faint	– ?	+ faint	+	
Eel grass (*Zostera marina*)	++	++	+ faint	–	–

It has frequently been noticed that with some algae a short exposure to light caused oxygen production which stopped on longer illumination, possibly due to the fact that the light was too intense. This observation requires further investigation to make certain of the explanation.

Another point of interest is that oxygen is produced as quickly as the lamp can be turned on and off and I can confirm BEIJERINCK'S statement that striking a match is sufficient to cause photosynthesis. The very rapid appearance of oxygen outside the cells of the plant, where it is detected by the bacteria is only further evidence of the rapidity with which oxygen passes the plasma and cellulose membrane of the cells (**2**). Oxygen is a very freely penetrating substance.

Fucus vesiculosus gave the best light of all. It was, therefore, placed in the dark with an emulsion of luminous bacteria through which a slow current of hydrogen was passed. The hydrogen was conducted over red

hot platinized asbestos, thus rendering it absolutely free of oxygen, and led to the bacteria in a lead tube sealed to glass with DE KHOTINSKY'S cement. If luminous bacteria are to be kept in good condition in absence of oxygen, the CO_2 must be largely driven off and this was accomplished by the hydrogen. After 2 hours the *Fucus* was illuminated and found to photosynthesize since the bacteria glowed. After 2 more hours in oxygen-free darkness the *Fucus* was again illuminated and found to photosynthesize again. Since the luminous bacteria reduce methylene blue in a few minutes and indigo-carmine in about one hour we can state that *Fucus* (and many other algae) can produce oxygen from CO_2 under complete anaerobic conditions, even after 2 hours anaerobiosis.

The only form which showed any stopping of photosynthesis in absence of oxygen was the eel-grass. Even this plant could photosynthesize in absence of oxygen after 15 minutes, but not after 30 minutes more. I am, therefore, led to the conclusion that the first step in photosynthesis in marine algae and in eel-grass does not require oxygen. Whether sugar or starch is formed in absence of oxygen I cannot say.

Summary

Fourteen species of marine algae, including members of the green, red and brown groups, are able to produce oxygen from carbon dioxide when illuminated in complete absence of oxygen.

The oxygen appears within a second after illumination, showing the ready permeability of the plasma and cellulose membranes to this gas.

Luminous bacteria are recommended as a test for oxygen under all conditions where they will survive.

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A STUDY OF THE EFFECT OF H-ION CONCENTRATION ON THE GROWTH OF *AGARICUS CAMPESTRIS* *

DONALD FREAR, J. F. STYER AND D. E. HALEY

(WITH ONE FIGURE)

Introduction

It has long been known that *Agaricus campestris,* the common mushroom, grows well on horse manure which has been partially decomposed. In order for fruition to occur, however, the manure must be covered with a thin layer of soil shortly after a good mycelial development has been obtained in the manure. Under these conditions mushrooms develop and mature over a period of several months. At the end of that time, however, the production ceases and before the medium has been depleted of nutrients. The cause of this may be the exhaustion of some food or food material, a change in the physical substratum, the formation of a toxin, or a change in the reaction of the medium. Then again it may be due to a combination of these and possibly other factors. In order to make a study of any single factor affecting the growth of mushrooms, it is highly desirable, if not imperative, to grow mushrooms in pure cultures.

Very little investigational work has been done on the growing of mushrooms in artificial media. Several French investigators, notably BOYER (1), have grown the mycelium on agar containing plant decoctions. BOYER found that the best growth was obtained where carrots, finely ground, were used as the basis of the culture. DUGGAR (2), and FERGUSON (3) also successfully grew mushrooms on synthetic cultures of known composition, although they did not attempt to carry their experiments any farther.

The object of this experiment was to determine, if possible, the optimum H-ion concentration for the growth of the mycelium of *Agaricus campestris* and to determine the effect of the organism upon the reaction of the nutrient medium.

Experimental

The medium used for the growth of the mycelium of the mushroom in question was that used by STYER (4), who, in cooperation with Dr. TRUE, of the University of Pennsylvania, found that a fair growth could be obtained with the following mixture:

* Approved by the Director of the Experiment Station as Technical Paper no. 440.

Black filter paper (Whatman's no. 29)	10.0 gm.
Casein	0.6 gm.
20 cc. of the following solution:	
$MgSO_4 \cdot 7\ H_2O$	0.02 M.
KH_2PO_4	0.04 M.
K_2SO_4	0.01 M.
$FeSO_4 \cdot 7\ H_2O$	Trace
$CaCl_2$	Trace

The differences in hydrogen-ion concentration were obtained by adding varying amounts of acid (H_2SO_4) and base (KOH). One set of cultures (P) was made up using 10 grams of the black filter paper plus 20 cc. of distilled water.

The culture medium was placed in 300 cc. Pyrex Erlenmeyer flasks and the flasks plugged with cotton to avoid contamination, after which they were sterilized in a steam autoclave at 15 pounds pressure and 120° C. for 30 minutes. The flasks were then inoculated by introducing a small piece of mycelium from a pure culture growing in manure. The check flasks were treated in the same manner as the others, except that they were not inoculated.

After inoculation the flasks were removed to a light-proof incubator, which was kept at 27° C. by means of an electric thermostat. The humidity was preserved by means of pans of moist sphagnum moss, in which the flasks were placed. Observations were made and the growth recorded each week in terms of the diameter of the colony in centimeters. The flasks were uniformly 8 cm. in diameter at the base.

The media were extracted with 50 cc. of distilled water, and the H-ion

TABLE I

COMPARISON OF THE INITIAL PH OF THE MEDIA WITH THE FINAL PH

Culture	pH	
	Initial	After 4 weeks
C	3.4	3.5
D	3.8	4.6
E	4.1	5.3
F	4.5	4.7
G	5.1	5.0
H	5.5	5.4
I	6.0	4.0
J	6.3	4.3
K	6.7	4.7
L	7.0	5.6
M	8.0	7.6
P	7.0	5.0

concentration of the resulting solutions were obtained by means of the potentiometer, using Bailey electrodes.

The data given in tables I and II were the averages obtained from three cultures grown under exactly the same conditions. The curve of growth with change in pH is shown in fig. 1.

Summary and conclusions

From the foregoing figures it may be seen that the best growth was obtained in the cultures which were nearest to a pH of 6.0, although the organism was tolerant to all conditions which were imposed upon it. The cultures in which the growth was greatest changed the reaction of the media

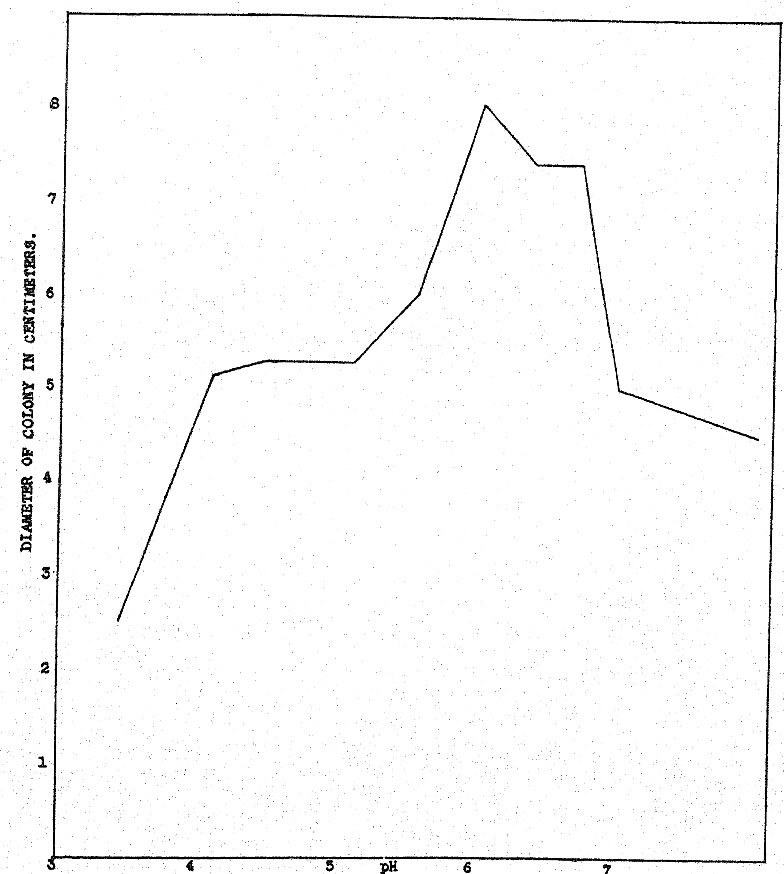

FIG. 1. Growth of *A. campestris* in relation to the H-ion concentration of the medium.

TABLE II

GROWTH OF *Agaricus campestris* AS AFFECTED BY THE H-ION CONCENTRATION OF THE NUTRIENT MEDIUM

CULTURE	pH	GROWTH (DIAMETER IN CM.)			
		1 week	2 weeks	3 weeks	4 weeks
C	3.4	0.4	0.8	2.0	2.5
D	3.8	1.0	3.0	4.2	4.2
E	4.1	1.0	3.0	4.2	5.1
F	4.5	1.0	3.8	4.7	5.2
G	5.1	1.2	4.4	5.0	5.2
H	5.5	1.4	4.6	5.0	6.0
I	6.0	2.0	5.0	7.0	8.0
J	6.3	1.9	5.3	7.0	7.4
K	6.7	1.4	5.6	7.0	7.4
L	7.0	0.5	3.6	5.0	5.0
M	8.0	0.4	2.0	4.0	4.5
P	7.0	0.5	1.0	2.0	2.0

to a marked extent, as evidenced by the marked difference in the initial and final pH in cultures I, J, K, and L.

The authors wish to thank Dr. R. H. TRUE, of the University of Pennsylvania, who suggested this work.

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EFFECT OF HIGH TEMPERATURE ON CHARACTER OF GROWTH OF CABBAGE

(WITH ONE FIGURE)

In a study of the rest period of cabbage, the writer found that full grown plants, which were removed to a greenhouse early in the fall, would not go to seed under relatively high temperatures. The plant shown in the figure was, with others, removed from the field October 8, 1925, and brought direct to the warm greenhouse in which the temperature averaged 70° F. This lot of plants was used as checks for comparison with others which were given a rest period. A similar lot was placed, for the same purpose, in the cool house in which the average temperature was 60°. None of the controls in the warm house developed seed-stalks but continued vegetative growth, and by March 7, 1926, all the plants had developed compact

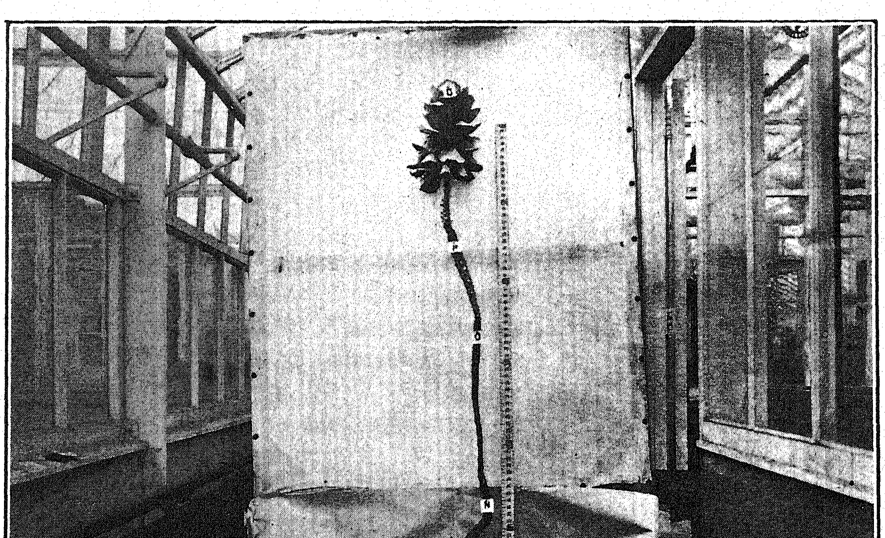

FIG. 1. N—Mature head, Oct. 8, 1925. O—Mature head, March 7, 1926. P—Mature head, Sept. 20, 1926. Q—Mature head, March 1, 1927, and a fifth head matured by September 15, 1927.

heads. The control plants in the cool house developed seed-stalks and flowers in 154 days. Similar lots of plants which had been allowed a rest period from October 8 to December 12 at a temperature ranging from 35°–45° F. were removed to the warm and cool greenhouses on December 12th. These plants, having had the above rest period, developed seed-stalks and mature flowers in the warm house in 39 days, while those in the cool house developed mature flowers in 68 days. Five of the heads were allowed to remain in the warm house for further observation.

About June 10th the heads cracked, vegetative growth continued, and by September 20th a third head had formed. By March 1, 1927, when the photograph was made, the plant illustrated had developed a fourth head. The fifth head was fully developed by September 15, 1927. The plant has been shifted to a pot containing new soil twice each year. At the present time it is growing in a wooden box 16 x 18 inches. As can be seen from the photograph, it is over six feet tall and at the present date it is at least eighteen inches taller than when the photograph was made.

The vegetative growth of cabbage plants in the warm house seems to agree well with the work on the beet (*Beta vulgaris* L.), by KLEBS, who states that it usually behaves as a biennial but that the plant may not be able to flower the second season if kept in the warm greenhouse during the winter. This cabbage plant is now over two years old, has produced five heads and at the present time (December 15, 1927) is growing vigorously. It seems evident from the growth of this plant that, with the control of the temperature, a plant may be caused to continue vegetative growth.—JULIAN C. MILLER, *Cornell University*.

NOTES

The Nashville Meeting.—The meeting held at Nashville last December demonstrated the growing strength and vitality of the American Society of Plant Physiologists. The membership was well represented in the attendance, and the programs were of good quality throughout the sessions. Much interest centered in the annual dinner for all plant physiologists, the evening of Dec. 28. Seventy-five members and friends of the Society were in attendance, and the dinner was made the occasion of a celebration of the 250th anniversary of the birth of STEPHEN HALES, and the 200th anniversary of the publication of his most famous contribution to botanical science, Vegetable Staticks. The interesting story of his life and works was given by the president as a feature of the after-dinner program. Following this sketch of HALES's life, was the official announcement of the establishment of the STEPHEN HALES Prize Fund, by BURTON E. LIVINGSTON. The main facts concerning this fund are recorded in another paragraph in this number of Plant Physiology.

The dinner was also made the time and place of the announcement of the second award of the CHARLES REID BARNES honorary life membership in the Society. This announcement was made by Professor FRANK M. ANDREWS, chairman of the award committee. These events made the occasion memorable, and those who were fortunate enough to be present have carried away the happy memories and inspiration of a very enjoyable meeting.

The Stephen Hales Prize Fund.—The effort to create a prize fund in honor of STEPHEN HALES met with a generous response on the part of the members and friends of the American Society of Plant Physiologists. The amount which had been contributed to the fund at the time of the meeting was slightly in excess of $1000. Gifts since have brought the fund to $1031.75, which is a very fine beginning. In order that an award might be made at the New York meeting in 1928, one of the most generous donors to the fund stipulated that $100 should be set aside, and kept uninvested for the 1928 award. This leaves $931.75 in the fund for investment. It has been decided to keep the fund open, so that additional gifts may be made. A number of individuals found it impossible to assist at the time the opportunity for contributions was given. Any one who would like to have a share in the building of this fund, has the privilege of making additions to it at any time by sending a check for any amount he desires to

give, to Dr. S. V. EATON, the secretary-treasurer, Dept. of Botany, University of Chicago. As the fund increases it may be possible to award a STEPHEN HALES Prize every year, or to increase the amount of the award if it remains a biennial occurrence.

The task of devising methods of handling the funds and making the awards is in charge of a committee of which Dr. JAMES G. PEIRCE of Stanford University is chairman. The other members on the committee are FRANCIS E. LLOYD, E. J. KRAUS, J. B. OVERTON, and B. E. LIVINGSTON. The establishment of the STEPHEN HALES Prize Fund on such a substantial basis reflects the earnestness, vigor, and enterprise of this organization.

The Life Membership Award.—The second award of the CHARLES REID BARNES honorary life membership in the American Society of Plant Physiologists was made to Professor FRANCIS E. LLOYD, MacDonald Professor of Botany in McGill University. An account of the award appears in Science for March 19th. Professor LLOYD has been a very able investigator in the field of plant physiology, and has made many worthwhile contributions to our knowledge in this field. During recent years his studies of the fluorescence of plant pigments, the physiology of conjugation in *Spirogyra,* and the habits of *Vampyrella* have attracted wide attention. His moving pictures of gametic fusion, contractile vacuole activity, and feeding habits of *Vampyrella* have been entertaining and instructive features of the annual meetings of the Society for several years. The American Society of Plant Physiologists, in this award, has expressed its sense of appreciation of his valuable services. Professor LLOYD forms the second member in the living link memorial which was established in honor of BARNES at the Kansas City meeting in 1925. The two members thus honored are:

BURTON E. LIVINGSTON, Johns Hopkins University,
FRANCIS E. LLOYD, McGill University.

Sixth National Colloid Symposium.—The sixth national colloid symposium will be held at Toronto, Canada, June 14–16, 1928. The guest of honor this year will be Professor WILLIAM B. HARDY, of the University of Cambridge. These meetings are always valuable, and any one who desires to attend is welcome. A small registration fee is usually charged, to cover the cost of certain of the social features. Plant physiologists have in the past found these meetings very much worthwhile.

Agronomy Meeting.—The Corn Belt Section of the American Society of Agronomy will hold the summer meeting at Columbus, and Wooster, Ohio. The meeting will begin at Columbus on June 21, 1928. In the late afternoon the scene will be shifted to the Experiment Station. Those in

attendance will be taken by auto to Wooster, where the meetings will continue on June 22 and 23. Last year the meeting was to have been held at Columbus, but the meeting had to be postponed because of other meetings.

Errata.—The attention of members and subscribers to Plant Physiology is called to the list of *errata* published at the close of the table of contents in the October, 1927 number. The editor regrets the occurrence of these errors, particularly those in connection with the citation of literature in one of the reports of the committee on methods of analysis. It is suggested that readers enter these corrections at the places where the errors occur. All members are invited to assist the editor in detecting and correcting any errors which are found in text or tables of papers published in the journal.

Program Committee.—The program committee for the New York meeting in December, 1928, consists of the following members: Professor D. R. HOAGLAND, University of California, chairman; Professor G. N. HOFFER, Purdue University, and Professor WILLIAM SEIFRIZ, University of Pennsylvania. The secretary-treasurer, Dr. SCOTT V. EATON, the University of Chicago, is *ex-officio* a member of the committee. Members of the society can cooperate with the committee by offering their best contributions for the program of the New York meeting. This will be the fifth annual meeting of the Society. May we not make it an outstanding meeting?

Investigations on Chlorophyll.—This splendid contribution by WILLSTÄTTER and STOLL has been given English translation by Dr. FRANK M. SCHERTZ and Dr. ALBERT R. MERZ, both of the Department of Agriculture. The book should appeal to many students, as the original German is not always easily read. The book has been printed on excellent paper by the Science Press Printing Co., Lancaster, Pennsylvania, and is nicely bound in cloth. As the book has been published by the senior translator on his own capital, members of the Society can show their appreciation by ordering a copy of the work, and placing copies in the libraries of our respective institutions. As it can be purchased only from the senior translator, his address is given in full. The price is $4.50, and can be had from Dr. FRANK M. SCHERTZ, 1305 Farragut St., N. W., Washington, D. C.

Physical Chemistry and Biophysics.—This book has been prepared for students of biology and the medical sciences, by Dr. MATTHEW STEEL, Professor of Biological Chemistry in the Long Island College Hospital, Brooklyn, N. Y. There are thirteen chapters, the first of which is a brief introduction. The chapter headings include the following: The nature

and structure of matter; general properties of matter; energy transformations in living matter; general nature of solutions; water, the greatest solvent; diffusion and osmotic pressure; the nature and behavior of electrolytes in solution; chemical equilibrium and the law of mass action; measurement of hydrogen-ion concentration; the colloidal state of matter; catalysis and velocity of chemical reactions; and dynamical physical chemistry of the cell.

The book is well written, and will be a very valuable aid to those who need the fundamental knowledge of the physical and chemical dynamics of life. It is more than usually helpful, and should have a warm reception among plant physiologists. The price of the book is $4.00, and is published by John Wiley and Sons, New York.

Biochemical Laboratory Methods.—At the time of his death, the late Professor CLARENCE A. MORROW, of the University of Minnesota, left the unfinished manuscript of a manual of Biochemical Laboratory Methods. This work has been brought to completion with the aid of Drs. R. A. GORTNER and T. A. PASCOE, and has been published for Mrs. MORROW by John Wiley and Sons. The experiments are arranged in nine chapters: The colloidal state; physical chemical constants of plant saps; hydrogen-ion concentration and buffer action; proteins; carbohydrates; glucosides; fats and allied substances; enzymes; and plant pigments. There are 233 experiments outlined, and any student who masters the methods given in this book will be a well trained biochemist. The directions given are clear and detailed. This is another book which can be recommended to the plant physiologist for his private library, and is not too expensive. The price is $3.75, and may be ordered from John Wiley and Sons, New York.

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STUDIES IN PERIODIC PRECIPITATION

F. E. LLOYD AND V. MORAVEK

(WITH FIVE PLATES)

Introduction

Much has been suggested or asserted concerning the significance of periodic precipitation in the interpretation of various biological phenomena, such as markings of surfaces, etc., and it is unnecessary here to do more than mention the names of LIESEGANG, LEDUC, KÜSTER, KLEBS (**6**), BECHHOLD to recall the discussions following this direction.

But it can hardly be doubted that, valuable as the pioneer work has been, all these suggestions have been tinged, if not deeply dyed, by vagueness or obscurity, so much so as to arouse skepticism as to the value of, or antagonism to the inferences drawn. Nevertheless a residuum of feeling remains that there is something in it all. The stripes of a zebra may be held to be produced in some other way than by periodic precipitation, but attentive consideration of these and similar remarkable productions generally leads to the admission that they look most suspiciously like some of the results of periodic precipitations; and more especially when the irregular or anomalous results of physicochemical experiments are contemplated. Of the latter, some illuminating examples will be given in the proper place.

It is patent that we know so very little about the physical phenomenon itself that the search for analogies is not at present very far justified. This is hardly surprising when we consider that it has been studied chiefly, when seriously, by persons more apt in physical experiment than in biological observation and interpretation. In their singleness of purpose they have sought out the simplest conditions for study, and this from their point of view is right and proper. But biological phenomena are overwhelmingly complex, and it happens that the more complex non-living systems are highly suggestive to the biologist. For this reason is justified their study,

which more than frequently uncovers problems to the physical chemist which otherwise might escape attention. It was the desire to explore this interesting field from the biological point of departure that prompted the authors, aware of both these values, to start on a perhaps perilous course. They emphasize the undeniably extreme complexity of living, and for that matter, non-living biological media, and the fact that it is in these that diffusions and the meetings of substances must take place. Further, unless it can be shown that there has been developed along the ages, means to prevent the interaction of colloids and crystalloids so as to produce periodic precipitations, these must in fact occur, however they may be obscured by the circumstances of their production. It can also be added that, even in non-living systems, patterns may be formed which, in the absence of known conditions of formation, and because of lack of periodic character, would by no means be referred to the proper category of phenomena.

Earlier observation on trichomes

The senior author's attention was drawn to the occurrence of periodic precipitations in the trichomes of plants (*Pelargonium, Saintpaulia*) (7) when treated by a method (for the microchemical detection of potassium) which had been tested and discussed by several writers. This consists in subjecting the tissues or cells which are the subject of inquiry to the action of cobalt sodium hexanitrite. After thorough washing in ice cold water for a length of time supposedly sufficient to rid the material of all free cobalt reagent, ammonium sulphide is applied in order to blacken the potassium-cobalt salt, itself pale yellow. It is unfortunate that all cobalt in an adsorbed sodium cobalt complex also blackens under the same conditions, so that there is always a doubt as to the results obtained. This doubt was felt in connection with the testing of trichomes for their potassium content, since, after the above briefly outlined treatment, the advance of the ammonium sulphide along the trichome from the base to apex (or also in the reverse order if the tip of a trichome happened to be broken), produced rhythmic precipitations of remarkable regularity, LLOYD (7). ROUPPERT (11) has also been able to procure like results in the stinging hairs of *Urtica dioica.* For a review of this field see ROUPPERT (11).

The material shows that under the conditions employed, the precipitation lies against the cuticle and wholly within the cellulose wall, not in the lumen. It would appear that the necessary condition for this result is that the cobalt reagent is bound to, or absorbed by the whole cellulose wall, including the septa. The entering reagent diffuses freely along the lumen of the cell but into the wall less readily. The directions of diffusion from lumen to wall give gradients, the form of which must affect the position of

the precipitation, which, lying wholly within the cell wall registers these diffusion gradients. There can be no doubt that the entering reagent diffuses less readily in the wall than in the lumen, for the septa,[1] in trichomes provided with them, cause interruptions in the periodicity, this depending, it would seem, on the altered diffusion rates of both reagents. Since, however, the cobalt reagent is held more firmly by the cell wall, it seems that the effect here is chiefly on the diffusion rate of the ammonium sulphide entering reagent. The continuously steady gradient of periodicity in ROUPPERT's experiments (trichomes of *Urtica*) was due to the absence of transverse septa.

This is not the only irregularity met with. The rhythms in various cells of a series in a trichome may alter from time to time, in contrast to the surprisingly great regularity at other times. In spite of all, however, the periodicity is quite as definite as when occurring in glass tubes (figs. 6, 7) if not as regular (figs. 1–5, plate I). The differences, aside from these irregularities, are referable to the special condition within a trichome, in which the cuticle corresponds to the glass wall, and the cellulose to a lining of gelatine, and not a solid column.

In the several cells of a series in a trichome, however, the periodicity may continue harmonically, relatively at least, disturbed only by the presence of septa.

In many experiments we have made, a periodic system of blue (cobaltous hydroxide) rings was observed, preceding the advancing black precipitate of cobalt sulphide, referable to the concentration of and the more rapid diffusion of NH_3-ions. We observed no blue rings in the senior author's earlier experiments. The occurrence of these blue rings depends on the concentration of the cobalt reagent, since the concentration of the entering reagent $(NH_4)_2S$ was probably too low (about 0.5 per cent.). It was in the form of equal parts of glycerine and $(NH_4)_2S$ of the strength usually used for qualitative work. The internal reagent therefore was probably too concentrated. We must point out that these earlier experiments were purely qualitative, but serve to bring out the following facts: When compared with rings in capillary tubes done at the same time and with similar concentrations of the same reagents, the topography of the rings in the trichomes is seen to be quite peculiar to the conditions therein imposed. In their original form, that is, when first laid down, the rings had a curved outer surface touching the cuticle at the point of original deposition. In the region where the rings are crowded, and where the precipitation took place rapidly, the outer limb of the deposit remained curved (fig. 1,

[1] HAUSMANN (3) showed that the frequency of rings in tubes depended on the viscosity of the gelatin.

Lloyd (7)). Further up the trichome the rings became flatter bands, a greater extent of the outer limb lying in contact with the cuticle. Because of the density of the deposit nothing more than this could then be seen. After standing four years the black precipitate has partially disappeared, the denser portions remaining. One such trichome is reproduced in fig. 1, in which now can be seen the peculiar form of the deposit. Figure 3a was made by enlarging a portion of fig. 1, and fig. 3b from a part of fig. 2. The topography indicated can have no other meaning than that available in the relative densities of the precipitate, this being due to diffusion gradient during formation. During the interval of four years since the original preparation was made, it is probable that S-ions have disappeared through the cuticle, in which they must be soluble to some extent. In another case (fig. 2) the rings were very narrow and sharp, and, as in all cases, but still more evidently, confined to the cell wall[2] and touching on the cuticle.

During the past few months a student, Mr. A. B. A. Evans, has been repeating the senior author's earlier experiments, so far as the reagents could be duplicated. Working with *Pelargonium,* he also obtained periodic precipitations. The character of these precipitations was visibly discontinuous, in some cases quite obviously so. They consisted of minute to coarse maculae, sometimes arranged in longitudinal rows, and apparently departed somewhat from the character of true periodicity. We believe, however, that such aberrations do not invalidate the periodicity (fig. 5), but merely indicate that the conditions obtaining have induced the formation of larger droplets of metastable colloidal precipitate, a condition held important by Hedges and Myers (4), and seen by Möller (9). In the other cases (fig. 4) the apparently continuous bands are resolvable, under higher magnification, into a cloud of minute, discrete droplets, so that the difference between the two is one of degree. It is to be noted, however, that the disposition of the precipitate is to a considerable degree controlled by certain markings on the surface of the trichomes, in the form of minute longitudinal tubercles which influence the emplacement of the precipitate. This has been observed by Rouppert. It is not mere accident; the same thing happens when for any reason the cuticle happens to become longitudinally folded. In this way the formation of minute capillary spaces favors the formation of periodic precipitation, as Rouppert would say. We prefer to say that it is the approximation of surfaces which does this; and this interpretation applies also to the minute tubercles just mentioned.

It seems evident to us that in the case of trichomes a fundamental fact emerges, namely, that the cuticle furnishes a surface or surfaces on which

[2] The bands observed by Möller (9) were also in the wall.

is induced periodic precipitation. Local changes of form of the surfaces, such as pittings (to fit the minute tubercles) and foldings have an added influence in bringing surfaces into closer approximation.

Another easily overlooked feature of the precipitation bands in trichomes is the fact that contraction of the cellulose occurs in these regions due to the dehydrating effect of the precipitate. The trichomes appear then as if cinctured. The degree of contraction is not very obvious macroscopically in photomicrographs but is at once revealed on examining fig. 3b; ROUPPERT's fig. 7 shows it also. We have observed like behavior in the case of cobaltous hydroxide and of cobalt sulphide rings in gelatin.

Earlier observations on capillary spaces in tubes and between glass plates

The senior author made some attempts to reproduce in capillary glass tubes rings similar to those seen in trichomes, but failed for reasons which are now obvious. The tubes were filled with about 15 per cent. gelatin ("Gold label") mixed with a small amount of cobalt reagent. The ammonium sulphide was mixed with an equal amount of glycerine and the tubes were laid in the entering reagent. Two forms of periodic precipitation resulted (figs. 6, 7). The one, in four larger tubes, was characterized by biconvex lenticular discs of very great regularity of form apparently harmonically spaced, alternating with evident but obscure thin rings. In the smaller tubes, instead of regularity, irregularity resulted, not at first, but as the reagent penetrated from both ends toward the middle of the tube. The precipitate consisted in partial rings attached to the wall of the tube, those on the two sides of the tube alternating more or less harmonically. Broad flat bands (discs) are formed toward the end of the process, as the concentration at the middle of the tube becomes exhausted. We have observed this also in cobaltous hydroxide rings, which are discussed later.

There are many details of structure in these tubes which might be commented upon, but as they have stood for a long time, and since during the interval secondary changes due to dissolution of the precipitate have occurred, no further description is given. Original observation, however, disclosed the fact of the great regularity of the periodicity, the lenticular form of the bands and their attachment to the glass wall. The final band formed, at the middle point of the tube (in the larger ones), was always irregular in thickness and placed midway between the last members of the approaching series.

Experiments made by allowing the entering reagents to diffuse into cobalt-gelatin under a cover-glass afforded equally distinct rings traversed by a pointed cross-shaped pattern, having the apices of the arms at the cor-

ners of the cover-glass. This is an invariable accompaniment of diffusion beneath a square cover-glass. The irregularities in precipitation consisted in transition from circular (really only apparently so) into spiral as the center of the cover was approached; branchings (always seen under covers if periodic precipitation occurs); and patterns due to adsorption of precipitate on surfaces not only of the glass enclosure, but of bubbles accidentally occurring. The pattern within the gelatin was not identical with the pattern on the surfaces, a feature which later on we shall see is of great importance.

The presence of many and various irregularities leads us again to emphasize this feature. We have seen that peculiarities of structure in trichomes determine or modify the character of the patterns in periodic precipitations; similarly, the presence and the form of surfaces, even of the wall itself and the surfaces of bubbles, etc., both influence these patterns in tubes and parallel walled capillary spaces. Among these irregularities are spirals (HATSCHEK (**2**)), maculations, disparity of patterns as between precipitate in the body and on the surface of the vehicle, to mention no more. When these facts are considered it becomes easier to expect, but perhaps more difficult to recognize, analogies in the animal and plant.

It becomes evident that, starting with uniform concentrations of reagent in the gelatin, different patterns may arise; and this opens to devastating doubt the whole question of the availability of the cobalt reagent as a test for potassium. It was found (LLOYD (**7**)) that the entrance of the reagent into the cell was marked by membranous precipitations which could not be interpreted as anything more than precipitation patterns. We may now assert, in the light of a reëxamination of earlier observation and of others now to be surveyed, that such precipitations are in reality periodic, and, far from indicating the emplacement of potassium in the cell (MACALLUM (**8**)), show rather that, the cell being a complex of colloidal materials possessing numerous interfaces, patterns which arise from periodic precipitations must occur, and that these patterns may, indeed, be of great complexity.

The previously described experiments with capillary tubes (figs. 6, 7) gave such beautiful periodicity that the precipitations were made the subject of measurement. Diffusion of the entering reagent was from both ends simultaneously. We note the following characteristics displayed by eight such tubes. Four smaller ones gave precipitations which were irregular. The rings were frequently partial, as if broken into halves which were displaced into zigzag positions. If the separate sections could be joined together, a spiral, approximately, would result. The rings (or rather plates) were broad in these four cases, and, aside from the features noted,

were regularly periodical. Of the four (on the left hand in figs. 6, 7) which were very regular, one showed disc-like thickenings in the plates of precipitation, from which had also been built up irregular dendritic processes extending backwards, *i.e.*, toward the nearer opening of the tube or opposite to the direction of diffusion of the entering reagent (the third tube from the left, fig. 6). There are evident also two systems of precipitation figures which are both black at present. One of these two may have originated as cobaltous hydroxide but of this now we cannot be sure. This would be the system of fainter transverse stripes seen in fig. 6 (tubes 1, 2 and 4 from left). The more definite and pronounced system was measured, applying the scale to the projected images from lantern slides, by Mr. A. B. A. Evans. It was found that the results do not support the conclusions of Schleussner (**12**), who maintains that the increase in the intervals between rings follows a geometrical progression, but rather harmonize with those of Hausmann (**3**).

Bubbles

In experimenting with cobalt sodium hexanitrite we found great difficulty in avoiding the formation of bubbles, and consequently labored under a feeling of frustration until we made a virtue of necessity and used the bubbles to enable us to further our interpretations.

These are bubbles of gas (NO, N_2O and N_2O_3) arising from the decomposition of the reagent, which proceeds more rapidly at higher temperatures (room temperature), in lower concentrations of the reagent, and at lower concentrations of gelatin, all due to lowering of the viscosity. Light also hastens the decomposition and production of bubbles.

When a mass of gelatin is exposed to a reagent, swellings or shrinkings may occur, as is well known. If in the form of a column, and exposed, *e.g.*, to ammonia (as in our experiments), the proximal end of the gelatin column swells and may protrude from the tube (fig. 36, plate III) for various distances. What is not at once apparent is that the middle of the column swells more than the peripheral regions where it sticks to the glass wall. Still less apparent is the fact that this strain is communicated beyond the diffusion limit of the entering reagent to the whole of the column of gelatin. This would have escaped notice but for the fact that bubbles betray the strain and assume corresponding shapes. They often arise in contact with the glass surface and, especially in small tubes, appear attached to it. In consequence of strain they are secondarily stretched and distorted in the direction of the mouth of the tube, assuming beautifully symmetrical shapes (figs. 10, 12, 13). The amount of distortion depends on the position of the bubbles, those in the middle point of the tube (as-

sumed to be open at both ends) remaining spherical. In fig. 10, in which this condition is seen, it is to be noted that the bubbles, because of the amount of distortion, overlap, as seen more clearly in fig. 12.

As may be inferred, the total volume of the bubbles may be very great, and it must be pointed out that the resulting difference in volume of the gelatin is not equal to the volume of gelatin protruding. The total volume of the gelatin is therefore reduced, and internal syneresis must have occurred.

It is also evident that the strain set up at or near the open end of the tube is transmitted throughout the tube. This is demonstrated also by the anisotropy of the gelatin column when suffering the strain. A freshly prepared tube shows none. The bubbles[3] also set up local strains which become evident by the same means.

The syneresis which we have inferred to occur displays itself sometimes by local transverse shrinkage. In one instance the gelatin left the glass wall for a greater part of the periphery between two rings (cobaltous hydroxide). Along spaces thus produced the gas which forms may travel toward the open end of the tube and escape. The evolution of gas becomes greater in the older parts of the column and causes much disturbance. The great variety of surfaces thus induced plays havoc with the regularity of precipitation, due to locally retarded diffusion, and causes most interesting irregular patterns to arise (*e.g.*, fig. 16).

The transmission of strain in a column of gelatin can very easily be seen in a gelatin-cobalt reagent complex in test tubes (fig. 9). Syneresis taking place, the additional strain is in course of time equalized by tearing of the gel. This begins naturally at a bubble and the cavity then becomes enlarged by conchoidal fracture. This is better seen when only a few bubbles are present (fig. 8). By regularly repeated fracture in one direction, long bubbles are obtained which have all the appearance of periodicity (fig. 11). One can see, however, that the tearing is merely a result of regularly repeated spasm caused by steadily increasing strain. It is a special case of conchoidal fracture, such as is readily seen in gelatin when it undergoes internal syneresis (fig. 8). Remarkable examples of such fracturing in gelatin-cane sugar in approximately saturated solution have been procured on a larger scale and we are confident that this behavior cannot be relegated to the realm of true periodicity. The strains, it is true, might result from periodic alterations in water content, but of this we have no certain knowledge.

[3] The gas in the bubbles is under a low pressure, for if a part of a column of gelatin containing bubbles is removed, the thinner wall, if external, collapses. If now the bubble be punctured under fluid, this rushes in and a smaller bubble remains inclosed in the fluid which has entered.

When conchoidal fracture intervenes there is usually evidence of twisted strain, in consequence of which a bubble may twist on its axis during growth and may even become definitely spiral. One here wonders how far strains in gelatin may play a part in producing the spiral precipitations noted by HATSCHEK (*l.c.*).

The presence of a bubble occupying a large portion of the cross section of the tube has disturbing effects on the direction and rates of diffusion. There occur consequently displacements of periodic precipitations resulting in twisted, broken and otherwise altered rings or bands. These will better be considered further on, it remaining now only to reiterate the importance of the surfaces introduced by bubbles. We find that the surface of a bubble lying in the path of diffusion affords the place for the accumulation of precipitate as part of the periodic system produced; while the bubble as a whole, being an impediment in the diffusion path, diverts the diffusion currents so that stream lines are set up analogous to those in air or water caused by relative motion of a body.

Periodic precipitations of cobalt sulphide (CoS)

When cobalt sodium hexanitrite is mixed with gelatin and placed in contact with ammonium sulphide, a black precipitate of cobalt sulphide is formed. This forms a barrier in the gelatin through which ammonia permeates more rapidly than S-ions. In consequence, when tubes, etc., are loaded with the cobalt-gel complex, the ammonia of the entering reagent moves forward more rapidly and produces in advance of the black periodic precipitation of CoS, a periodic system of blue rings of basic $Co(OH)_2$ if the concentrations are high enough. We shall consider the sulphide precipitate first, but must speak of blue rings at the same time. We leave more detailed consideration of the latter till later.

We have studied the character of the periodicity in the black precipitate (as we shall hereafter designate the CoS) in capillary, medium sized, and larger tubes. The inside diameters of the tubes were 0.37, 0.54 and 0.57 mm. for the small, 3.62 mm. (two long tubes), 2.96–3.40 mm. for a quartette of tubes, 7.1 for another, and test tubes 12 mm.

During the earlier period of diffusion, the precipitate formed is for some time apparently homogeneous (figs. 14, 16). It is usual to say of this condition that no rings have been formed, and the inference is that periodicity has not as yet occurred. This, however, is not the case, since if the column swells and protrudes from the end of the tube, the rings or bands become apparent (fig. 36). The periodicity in the tubes in fig. 6 is visible, at the outer ends, only under the microscope. The failure to produce even microscopically visible periodicity is, it seems, no proof that periodicity is absent.

Again, after long standing, secondary changes occur in the black precipitate which betray the periodic structure. Before any periodicity becomes obvious, examination by reflected light properly adjusted will reveal to the eye the zonation on the inner glass surface (fig. 32). Later the zones become quite distinct (fig. 33) until they become separated into groups of rings. The outer end of the gelatin column, which projects into the vessel containing the entering reagent, at length breaks up into these groups, being set free by the dissolution of the binding gelatin (figs. 64, 69, plate V). By study of portions of the column which have lain in ammonia for some time (fig. 69), we can see quite clearly the composition of the system, which is obscure and only partly decipherable when still in the tube. Before revealing the structure so seen, we must examine the periodic precipitation arising from the more rapid entrance of the NH_4-ions.

Ahead of the front of the black column arise blue rings and discs, the Saturn structure of Popp (**10**). In positions where these are sufficiently separate for observation it may be seen that the black column gradually advances beyond, let us say, a given blue ring. At about this time the next blue ring, which is later to be overtaken gradually darkens. After being overtaken it continues to darken till it becomes dead black, so that it can be recognized within the black column by its contrast in this regard (figs. 32, 33 to be compared with fig. 21). The cobalt sulphide precipitate is relatively grayish and can be recorded photographically. As the column advances, or also with long standing, the black column included between each two blue rings becomes resolvable into several rings. The number of rings at first appears to be determined wholly by the numbers and positions of the blue rings, there seeming to be a necessary relation between the two sets of periodic precipitations. In order to test this we produced a periodic system in gel-cobalt sodium hexanitrite, with ammonia as the entering reagent (fig. 39, plate III). After the precipitation was complete, we substituted ammonium sulphide for ammonia upon which we observed the dissolution of the blue rings as they were overtaken by a deeper brown *clear* zone, considerably in advance of the front of the black precipitate.[4]

The rings in the black had a much higher frequency and bore no relation numerically or spacially to the erstwhile blue rings. There is, therefore, no necessary relation between the two systems. In the experiments recorded in figs. 14–22, plate I, and 24–31, in plate II, however, the conditions were such that the blue rings never disappeared. They merely blackened and later retained their identity, so much so that they were found intact in the vessel containing the ammonium sulphide entering reagent several

[4] As to the chemical nature of this clear zone, we cannot at the moment decide. It is perhaps to be considered as red $Co(OH)_2$ formed in a higher concentration of newly entering ammonia from the $(NH_4)_2S$.

days after the swollen column had been expelled from its tube. Pieces of the gelatin column containing the two systems of rings thus obtained (fig. 69) were gently heated to see if the gelatin had been altered in regard to its softening point. We found that the black rings dissolved at once, but the blue rings remained intact (fig. 69). The color of these intact rings in strong light was distinctly bluish, though casually to the eye appearing black. We interpret this as indicating the adsorption of the black on the blue salt. A certain disturbance of the spacial arrangement of the black rings, due to the previous presence of the blue, has been observed; and this would, if adsorption occurs as indicated, be inevitable. Such delicate rings are very fragile and may be sustained merely by the continuity of precipitate and not by the gelatin. We should here note that the blue rings shrink shortly after formation (fig. 22), and there is no doubt that the shrinkage results from a dehydrating effect on the gelatin; this so contracted zone remains relatively incapable of responding to the swelling effect of ammonia.

The black rings have a ring-and-disc structure (fig. 66), precisely that "Saturn-structure" described for blue cobaltous hydroxide by Popp (**10**). We found this to be true also of the precipitation (fig. 38), when procured in large tubes (fig. 35).

When first laid down, the black precipitate appears first in banded form, but the position of visible bands is not constant. In no case have we been able to observe the same number of discrete bands as subsequently become visible; these usually appear to the number of one or two, while subsequently five or six appear (fig. 69). Later, on standing, whitening of some rings takes place (fig. 32). This may be due to a lower cobalt sulphide content or to secondary changes or changes by condensation toward the middle of the rings and dilution of CoS in the spaces between. This distinction disappears with time and we then find uniform black bands of ring-and-disc structure.

Curious irregularities occur. Partial discs are common, and we have found remarkable dendritic or vermiform black masses of precipitate traversing the bands more or less longitudinally with respect to the tube.

After the black precipitate is laid down, a condensation of the gelatin involved occurs as in the case of cobaltous hydroxide, but to a lesser degree. On subsequent swelling in the presence of ammonia the zones of gelatin containing the precipitate do not swell as much as the unoccupied gelatin, so that the freed column shows constriction at the black bands (fig. 69 when examined with a lens). This we have already mentioned as occurring in trichomes, as Rouppert also observed. This shrinkage is visible in the tube and becomes so because the total effect of black zone shrinkage is to pull the gelatin away from the tube wall, thus causing reflecting surfaces.

Blue rings

Three large tubes (12 mm. diameter) were charged with 10 per cent. "Difco" gelatin, and 1 per cent. $Co(NO_3)_2$ with 21 per cent. ammonia diluted 1:5, 1:10 and 1:20 as entering reagent. Constant temperature was maintained at 11° C. (fig. 40).

For several days, although periodicity became evident, it lacked regularity. Bands were to be seen but they were sometimes vague, and when more distant, showed no harmonic space relations. During this time the advance of the front of the column proceeded at the following relative rates, the last column being the record at the end of four days.

a (1:20)	19	26	38
b (1:10)	24	32	46
c (1:5)	27	37	54

That is, the rates vary with the concentration but not in constant proportion. After several days, distinct and harmonically spaced rings appeared, earliest in the lowest concentration of entering reagent. Meantime the uppermost zone of the gel became colored red (red cobalt hydroxide) the entering reagent dissolving the earliest formed blue precipitate. In this red region there is no visible periodicity. The extent of this reaction varied with the concentration of entering reagent. The distinct bands had the ring-and-disc structure, "Saturn structure" mentioned by HATSCHEK, described by POPP (**10**) (figs. 66–68). That is, a thin ring or band is adsorbed at the gelatin-glass interface, the disc being a circular plate laid down within the column of gelatin in approximately the plane[5] of the ring. It is difficult to separate the band from the glass. We succeeded in separating a portion of the column and obtained longitudinal sections which display the Saturn structure. It is suggested by these figures (67, 68) that the gap between the ring and disc becomes secondarily filled by the spreading of the precipitate.

The ring-and-disc structure was obtained in beautifully regular form in two smaller tubes (figs. 14–21) 2.25 mm. inside diameter, filled with "Difco" gelatin 10 per cent. and 5 per cent. cobalt reagent (fresh) with aqueous ammonium sulphide 1:10 as entering reagent. Both ends of one tube and one end of the other gave like results. Having been unable at this time to repeat this experiment with similar results,[6] we applied entering reagent to the remaining end, which had been kept sealed by a rubber tube,

[5] We are at the present writing able to state that in general the disc lies in front of the equator of the ring, though whether or not this is true of the earliest ones formed is uncertain.

[6] This has now been done; see later.

and obtained similar, but not, in time, quite identical results. A possible explanation lies in the physical changes (probably syneresis) which had overtaken the complex during the interval.

In these experiments the advancing black precipitate was preceded, as always, by a broad zone of clear yellow color indicating migration of cobalt ions from this region, within which are laid down rings and discs with great regularity so long as the homogenity of the medium (gelatin) remains undisturbed.

During the earlier period, the periodicity is scarcely observable; however, it is doubtful that the first precipitate is truly continuous but is rather only apparently so. Soon, however, a denser central and peripheral region become visible which gives place to distinct periodicity, and each band is then composed of a ring and disc. These may be laid down simultaneously (so far as observation will permit us to say) or, as a matter of undoubtedly correct observation, the ring is sometimes at least laid down *before* the disc (fig. 19).

As the precipitation column develops, the discs become smaller until they are no longer formed; or, as may occur, they may be formed anew to the number of one or two, and sometimes we find small granule-like points or larger discs formed. The presence of a bubble may cause such irregularities to occur, and the disturbance so caused may lead to the formation of incomplete discs (fig. 14) but it may also have no obvious cause. Discs, for this or other reasons, may have perforations or embayments (figs. 64, 70) or they may, less frequently, become connected with the ring by an isthmus. Similarly the discs are not equally thick, nodules or fenestrations occurring. All of these facts must have a bearing on any explanation of the phenomenon of periodicity. We did not find it easy to duplicate the ring-and-disc structure but succeeded finally by using 20 per cent. gelatin with 0.5 per cent. $Co(NO_3)_2$ in tubes 2 mm. in diameter with ammonia 1:10 as entering reagent. Within an hour the ring-and-disc structure was visible. The blue precipitate was preceded by a pink zone 1 mm. thick. Beyond this could be seen, by careful illumination, the well-defined face of the diffusion column, quite colorless, and made visible photographically by adjustment of black and white background.

When the tubes used were of capillary dimensions (0.54–0.56 mm.) we obtained only rings, or occasionally plates arising by extension of the ring across the lumen (fig. 37). The bridging of the columns of gelatin may therefore take place by fusion of disc and ring or by thickening of the ring alone. These facts cannot be contemplated without realizing the importance of the glass-gelatin interface in furnishing a footing of fixation for the precipitate in our experiments. The rôle of bubbles in this regard and

in varying the topography of the patterns formed has already been discussed. We were therefore led to test further the possible effect of surfaces on pattern by making up a cobalt-gelatin mixture, introducing thereinto (a) a glass rod running approximately along the axis of the gelatin column; and (b) a lot of glass fragments. The different ends of the tubes were exposed to ammonia and ammonium sulphide. It was difficult to observe what was happening to the rod while in position; that is, difficult to separate reality from reflection. But upon removing the tube after sufficient time had elapsed we found both blue and black precipitates on the surface of the rod (fig. 60). The patterns formed were quite bizarre, and would not, we believe, be regarded as the product of periodic precipitations. While we are justified in regarding the pattern on the rod to be correlated with that on the tube wall, it may be that the forces at play, while strong enough to start the precipitation, were insufficient to locate it in positions of strict correspondence. The patterns on the wall of the tube also showed much irregularity (figs. 58, 59), more indeed than would be expected, but while this may be connected with the presence of the glass rod, it seems unlikely.

In the presence of glass fragments, rings of fair regularity were formed on the tube wall (fig. 62). Less internal precipitation occurred than was expected, but when it did, it always originated on the surfaces of glass fragments and secondarily bridged over from one to the other (fig. 63).

Again, the question of the character of the surface has arisen in the past. We found that the behavior of these reagents remained indifferent to the difference of surface as between quartz ("Vitreosil") and glass.

Just as, in the cases before us, surfaces play a part in fixing periodic precipitations, so the displacements of the medium by bodies affording surfaces provide conditions for the unequal emplacement of reagents, and for changes in the directions of diffusion. In consequence, the periodic bands become displaced, and various twistings and changes in their dimensions take place. The rings seen at and above the bubble in fig. 21 are a clear example. The effective concentration of reagents was attained at an abnormal position, nearer to the last formed ring than otherwise would have occurred. The next formed ring was twisted, and the second following had a gap. The part of this ring that should have occupied the gap was formed further along the tube in a position corresponding to the oblique displacements in the preceding rings. It is evident that the bubble had caused an oblique redirection of diffusion.

Similarly, the dimensions of rings are affected by directions and relative rates of diffusion. In a homogeneous medium (as much as we questionably suppose gelatin to be) the farther the entering reagent has to travel, or, the greater the effective lowering of concentrations, the wider the rings

become (fig. 65). A sufficiently large bubble suitably placed will interfere with the diffusion of the reagents so that an unexpectedly broad ring will occur. If the bubble nearly blocks the column symmetrically the ring occurs at or rather just beyond the zone of smallest cross section (fig. 65). It is probable that such a bubble affects the positions of all the rings of the system.

The width of the rings, in addition to varying uniformly, may vary in any particular case. An individual ring may thus become nodular, and nodes corresponding in position may occur on neighboring rings (figs. 24, 26, plate II). If close enough together these nodes become confluent and a network of square meshes occurs (fig. 23a). Sometimes the transverse elements are so far suppressed that only nodes and their longitudinal connections ever occur. Thus, instead of rings, longitudinal lines of precipitate result (fig. 24). All sorts of intermediate confusions of pattern arise from these aberrations, and the consequent patterns, *e.g.*, fig. 16, are very suggestive to the biologist; and to the physical chemist are examples of periodic precipitation in which the resultant pattern often not in the least reflects the periodicity.

We have already pointed out that the rings are laid down first at the gelatin-glass interface. The ring is at first a flat band or ribbon but in a short time it begins to contract and becomes furrowed (fig. 22). Now it can be seen attached to the glass along two fine lines, the margins of the band. We have sometimes thought that the whole of the gelatin column shrinks, but whichever view is correct, the fact remains that the most complete attachment to the glass is usually along the two margins of the band (fig. 22). In any case the shrinkage of the gelatin appears to result from the presence of the precipitate. Displacements of a ring or other conditions may occur in such fashion that only one margin or a thin zone of the ring is attached to the glass. This we have seen in the presence of a bubble, and it can be seen in the region of the rather crowded broad rings in fig. 26.

The constitution of blue rings appears not always uniform. We have observed that, under some circumstances, blue precipitate is first laid down, followed by white precipitate as seen in fig. 23, which was from a photograph taken through a blue-absorbing filter. Here one sees a double white line bounding the photographically dark (= blue) ring. The white precipitate is probably a form of cobaltous hydroxide, in a fine state of dispersion and lower concentration but the use of the blue-absorbing filter shows that the white color is due merely to the state of dispersal of blue precipitate. These rings ultimately turn blue.

The cobaltous hydroxide and cobalt sulphide precipitates appear far more homogeneous and continuous than others we have observed, *e.g.*, silver

chromate. Examined ultramicroscopically, the former has much the optical appearance of a casein coagulum, and cannot at least be regarded except as a deposit of finely divided, probably metastable precipitate. The latter behaves, as we observed in preparations between glass plates, as other metastable precipitates, *e.g.*, sulphur, in which the smaller droplets disappear and contribute to the growth of the larger ones, which eventually crystallize. We thus obtained crystals of cobalt salts in some, and of sulphur in other preparations.

Although we observed transverse shrinkage in the gelatin column, we recall that the presence of the entering reagent (ammonia or ammonium sulphide) causes the proximal region of the gelatin column to swell. The amount of swelling is greatest along the axis of the column. Radially directed pressure must also exist, as appears from the swelling of the column on leaving the tube (fig. 36). If periodic precipitations are being laid down during the swelling, and the transmission of the strains due thereto are transmitted at a rate to overtake the bands (rings and discs) already laid down, the discs especially become displaced in the direction of the swelling, and are then dished (figs. 20–21). Misplacement may occur because of the presence of a bubble, that is, the disc may appear out of position either as a result of swelling, or from disturbances of diffusion gradients (figs. 17, 18). The strains set up by swelling can be seen, as above stated, between crossed nicols.

White rings

Pale white periodic precipitations of a fine, widely dispersed precipitate were first observed in a test tube holding gelatin only, with ammonium sulphide as entering reagent. The precipitate upon magnification was seen to be composed of minute dumb-bell-shaped, crystalline pairs. As crystallization advanced a second pair might arise at right angles to the original system, now forming a sort of cross (fig. 45). With later growth compound sphaerocrystalline masses resulted. They were determined to be sulphur crystals. This peculiar morphological form arises in gelatin—that is, in a watery colloidal medium—though it is not the only crystalline form so to arise.

Rhythmic crystallization of sulphur in this wise was obtained in the following experiments:

(a). A gelatin-ammonium sulphide mixture was placed in a series of tubes of various diameters and left open. The control was sealed. In all the open tubes periodic precipitation of sulphur was obtained. The figure (fig. 41, natural size) was made before a final ring was added but the preparation suffered from overheating in a horizontal position and so the

photograph of this is not reproduced. The periodicity of the rings was the same for all tubes. The precipitate was laid down as rings close to and in contact with the glass wall, and later filled, thus becoming a plate. The character of the precipitate made it difficult to see if the ring-and-disc structure obtained, though there seemed to be a concentration on the glass.

This precipitation may be the result of one of the following conditions:

(1) Diffusion of oxygen and the oxidation of sulphide to polysulphide and sulphur.

(2) Evaporation of ammonia, leaving H_2S and following the process mentioned in (1).

(3) Dissociation of sulphur from the protein by alkaline hydrolysis. Although white bands are made in like amounts in the tube whether sulphide is present or not in the entering reagent, in both cases it is due to the same cause, since, at the time of its formation in the tubes provided with $(NH_4)_2S$, it always occurs *beyond the limit of the black column,* and hence at a place to which the sulphide has not yet diffused. At high concentrations of ammonia the white precipitate remains longer than at low. In view of the studies of HOFFMAN (5) and GORTNER it seems probable that alkaline hydrolysis is responsible, though we note it to occur at room temperature. Further work is needed to decide this question.

(b). A gelatin-cobalt reagent complex (20 per cent. "Difco" gelatin, 0.5 per cent. cobalt reagent) with ammonia 1:50 as entering reagent, gave no precipitate. Only two successive darker yellow moving bands occurred. On substituting stronger ammonia (1:10) in the course of a week three rings of white precipitate, later followed by three additional rings, six in all (fig. 41, taken before the last ring was formed) were produced in the course of two weeks. The precipitate (fig. 45) was identical with that observed when sulphide was present (fig. 44). Since there was no source of sulphur present in the reagents, we must suppose that some part of the gelatin molecule was split off by the NH_4OH and sulphur set free. The same result was repeatedly obtained. As in the previous experiment the form of the crystal indicates an originally very finely dispersed metastable condition of the precipitate.

Effects of temperature on the above described periodic precipitations

BEHAVIOR AT LOW TEMPERATURE

Four tubes were prepared with gelatin-cobalt complex (20 per cent. "Difco" gelatin; 5 per cent. cobalt reagent). One end of each of the tubes of a pair was exposed to ammonia 1:5, the other to ammonia 1:20, and the

other end to ammonium sulphide 1:5 and 1:20 respectively. One pair of tubes thus prepared was kept for 100 hours at 3° C., another pair at room temperature. The following observations were made (figs. 24–31, plate II).

Diffusion in the cold is not as rapid as in the higher temperature. This may be due, in part at least, to the higher viscosity of the gelatin at lower temperature.

The lower temperature favors the formation of a white precipitate of metastable sulphur, yielding double sphaero-crystals such as have been described previously. If sulphur is present in the entering reagent, white precipitate is found in advance of the black and moves forward with time (figs. 27, 31). It finally becomes evidently periodic.

If only ammonia is present in the entering reagent a second general zone of white precipitate occurs near the mouth of the tube. Two periodic zones occur at lower and 3–4 in the higher concentration. These do not move forward (figs. 25, 29). After 100 hours at 3° C. this pair remained at room temperature, after which the inner zone of white precipitate gradually disappeared and blue cobaltous hydroxide appeared in the same position. At the lower concentration of entering reagent the blue rings (about three in number) are broad, vague and rather irregular (figs. 25, 27); at higher concentration they are more numerous and more sharply defined (figs. 29, 31). The white rings near the mouth of the tubes remained unaltered for a long time, and no blue rings appeared here.

Behavior at room temperature

The behavior of black and of blue rings at room temperature (figs. 24, 26, 28, 30) was in general as already described, except that at the concentrations used there were no discs formed, but rings only. These were wider and vague at low concentrations of entering reagent, and more numerous and more sharply defined at higher concentrations, at which also the distance to which the ring formation was carried on is greater. This distance varies with, but is not constantly proportional to, the concentration of the entering reagent. We note, however, an exception in tube no. 6, fig. 24, which we cannot explain.

Although NH_3-ions must have diffused to the position indicated by the white zone to the right of tube 3, fig. 29, no cobalt rings had been formed. Only as this white zone began to disappear did ring formation occur, and indeed after the tube had been brought into the warmer temperature a single delicate blue ring developed, followed at a distance by others. We see therefore that the entering reagent may move forward without causing periodic precipitation (*cf.* tubes 4 in figs. 28 and 29). This means that a

precise concentration of the reagents is required to cause the precipitate to form in a particular position, neither higher nor lower concentrations sufficing.

Experiments in which the reagents meet in a capillary space between glass plates

The gelatin-cobalt complex was placed between a slide and square cover-glass convenient for microscopic study. The preparation was laid in a petri-dish, and covered with entering reagent which could then diffuse from all sides of the cover-glass.

There was always observed in such preparations a diffusion pattern in the form of a cross with pointed arms, the points at the corners of the cover-glass (figs. 46–48, 54, 55). This "diffusion" cross demonstrates the movement of the reagent in the gelatin to meet the entering reagent. The dimensions of this cross are determined by the relative diffusion speed of the ions involved.

Evident periodicity may or may not appear (figs. 46–55, plates III–IV). While the exact concentration which will produce it is not exactly easy to state we find in general that too high concentration of either reagent was unfavorable.

The results depend also upon the kind of gelatin used; we obtained no visible periodicity till we began to use "Difco" gelatin, although in earlier experiments the senior author obtained rings in all experiments done with "Gold label" gelatin. We think also that the viscosity of the gelatin plays a part, for we found that a long period of setting in a cool temperature, which rendered the viscosity higher and diffusion slower, favored the occurrence of visible periodicity. This is shown also by the fact that, if the entrance of sulphide was preceded by ammonia, as evidenced by the formation of blue rings (fig. 54), the progress of the black precipitation (fig. 55) was more rapid. An experiment kept at first at a lower temperature in a refrigerator, and only a short time at room temperature, gave no blue rings, and a less well marked periodicity of black precipitate (fig. 46).

We have already discussed the relation of blue and black rings, showing that there is generally no relation between the appearance of the periodicities. In our cover-glass experiments, however, we observed exceptions to this, *e.g.*, in the experimental results shown in figs. 54–55, we find that an entire system of blue rings was first formed beyond the advancing edge of the black precipitate and that the pattern later formed by the black precipitate was identical with it. The absence of further black precipitate was due to low concentration of S-ions. We watched the progress of the black precipitate under magnification and found that the blackening of the

blue rings was the first step in the process, the building up of the black precipitate then extending backwards. The significance of this earlier blackening of the blue rings (common, as seen by all our experiments in which blue rings were formed ahead of the black) lies, we believe, in surface relations. The cobalt hydroxide already laid down offers an extensive surface for adsorption of the black precipitate. We shall show that the glass surface, in the absence of the blue precipitate, becomes of paramount importance.

It is not surprising, in view of these facts, that visible periodicity became, in our experiments, very well marked if we either acidified the cobalt (acid) reagent or rendered more alkaline the ammonium sulphide. Such results as are shown in figs. 48–50 were obtained.

The rings were very closely packed. In addition the experiments show the effect of the diffusion of Co reagent from the cross region, leaving here lower concentration. This is reflected in the reduced number of rings per unit distance (measured normally to the rings). The total distance occupied by the periodic precipitation measured along the diagonal of the square is greater than the distance normal to the rings where they are parallel to the side of the square. The total number of rings along the diagonal is greater than elsewhere, and this explains the articulation of rings along the lines of delimitation of the cross pattern.

The pattern of the rings is a function of the surface of the glass plates, and is relatively independent of the concentration (illustrated by the cross pattern, which expresses concentration). We thus obtained two patterns superimposed on each other, the cross pattern and that of the advancing rings. Fig. 48 shows well the patterns in question.

A proper consideration of this principle enables us to interpret the patterns arising when additional variously formed surfaces intervene in the path of a diffusing reagent, as, *e.g.*, when bubbles so occur. The simplest condition to contemplate is that of a spherical bubble (fig. 49).

Furthermore we have observed that always in the case of bubbles the surface plays the same rôle as the surface of a glass tube, *i.e.*, there is always on the surface a greater amount of precipitate corresponding to a band more or less removed. We thus encounter again the ring-and-disc structure (figs. 49, 51, 52, 53).

With respect to the ring between glass and cover, we must say the same; comparing the space between to the lumen of a tube, the distance acts correspondingly to the diameter of tube. If this distance is great enough we shall have a ring (or ribbon) and disc; if narrow, the ring alone may fill the space as in capillary tubes.

In order to test the effect of various kinds of surfaces, we introduced gypsum (figs. 51, 52) and oil (fig. 57). The gypsum reacts with ammo-

nium sulphide, forming calcium polysulphides. These react with the surrounding gelatin, which is thus rendered highly viscous and independent of the general mass; it then has the appearance of a yellowish vitreous mass, containing many irregularly scattered, partly dissolved gypsum granules. The surface of the vitreous masses served for the diffusion of periodic black precipitate, as also the smaller irregular granular appearing masses included. The resulting patterns, even when very irregular, yield to analysis in terms of ring-and-disc structure. This is quite obvious in fig. 51. However bizarre the patterns may be, we see no reason for doubting their periodic nature.

In the case of oil drops we observed an additional matter of interest, *viz.*: the crystallization of sulphur from the metastable black precipitate. These crystals were of metastable forms in the precipitate remaining (fig. 57).

Another feature of pattern is to be seen in figs. 53 and 56, in which the surface of gas bubbles is apparently folded and densely packed with black precipitate. It would here seem that the surface layer of the gelatin in contact with gas space is swollen, the effect of the ammonia, probably. The folding is purely a mechanical adjustment of the surface but we have seen evidence that the swellings are periodical in that they correspond to a periodic precipitation.

The importance of surface having impressed us, we have repeated ROUPPERT's experiment of using fluid cobalt reagent under a cover and allowing the sulphide to diffuse with it. He obtained visible periodicity, between which, however, and the repeated breaking of early formed precipitation membranes (ROUPPERT), we see no connection. We confirm ROUPPERT in his observation of periodic precipitation but we found that almost *identical patterns occurred on the two opposed glass surfaces,* the one looking through the microscope as if it were a "ghost" or reflection of the other (fig. 71). In order to be certain of our observation, we made a second preparation, washed away the reagent and removed the cover-glass; then we found almost identical periodic patterns on the two surfaces. Fig. 72 is the pattern photographed during the progress of precipitation. Fig. 74 is of the slide and fig. 73 of the corresponding corner of the cover-glass. We could watch the formation of the precipitate and the building up of the pattern.

Within the general area of the new ring one can observe the apotheosis of minute but visible granules which were free in the fluid as evidenced by their Brownian movement. Meanwhile a pale bluish zone was being built up on the glass surfaces. It seems that many much smaller and invisible particles of precipitate are being formed simultaneously. These must be-

come quickly adherent to the glass, since the larger visible particles suddenly stop their movement. This occurs evidently at the moment of becoming fixed to the surface.

That this precipitate is in a metastable condition is shown by the fact that clear circular areas are formed immediately when the layer is thin, these areas being caused by the evaporation of precipitate to form small crystals (fig. 71). This has been repeatedly observed in many preparations.

The peculiar pattern seen in fig. 72, where it appears scalloped, is due to disturbance of diffusion. These disturbances probably cause more or less local mass movement, unavoidable under the circumstances of the experiment.

Methods

The observations here presented were made in connection with experiments the chief purpose of which was orientation. The systems employed are admittedly complex.

The periodicities were studied (a) in glass or vitreosil tubes of various bore from 15 mm. to 0.5 mm. As we found no difference in the results obtained with glass and quartz, we have avoided further reference to this comparison; (b) in capillary spaces between glass plates (slides and square cover-glasses).

"Gold label" or "Difco" gelatin was used. The "reactant" was mixed with the gelatin and the mixture allowed to set, after which the entering reagent was added.

Smaller tubes were filled and the ends exposed to the entering reagent in a test-tube opened on the side, the tube being supported in position by a rubber stopper. The two ends of an entirely filled tube, if of sufficient length, could be thus exposed to different kinds or concentrations of entering reagent.

Glass plate preparations were exposed to the entering reagent in petri dishes, covered.

Photographic records were kept. Tubes were surrounded with water or glycerine to obviate reflections. Color screens were used to procure contrast. We find that a photographic record is of the greatest possible use in studying the protocols, as well as in illustrating the observed facts.

Summary

The banded precipitate of cobalt sulphide occurring in the trichomes discussed in this paper lies in contact with the cuticle and within the cell membrane. On comparison with precipitation in gelatin in small tubes, the walls of the tube—or the interface gelatin/glass—is found to correspond

with the cellulose/cuticle interface. Periodic precipitation may occur in the lumen of trichomes, but we have nothing further to say of these at this time.

The precipitate is laid down as fine or coarser granulations or droplets, doubtless in a metastable condition. These granulations become distinctly visible when there is a structural basis, such as minute tubercles, etc. This is due to the approximation of surfaces favoring local adsorption of the precipitate.

The explanation of the localization of precipitate was made clear by our subsequent experiments, in which we found that the surface of the inclosing vessel (the gelatin or water/glass wall interface) plays an important rôle in the emplacement of the precipitate. Thus arises (in all cases observed by us) a ring-and-disc structure, though this is less obviously the case when the precipitate is markedly discontinuous (*e.g.*, sulphur). It is appropriate to mention in this connection that R. B. PEEL, while a student with Professor STEWART YOUNG, found evidence of the adhesion of ammonium chloride in bands on the glass wall of the vessel in which the reaction of ammonia and hydrochloric acid had proceeded. This reaction occurs rhythmically. He speaks of the "catalytic action" of the wall of the vessel (in MS. Library, Stanford University). Further study of such precipitates is desired. It was found, accordingly, that the periodic precipitate of cobalt sulphide in the absence of a colloidal medium, observed by ROUPPERT, resides on the glass surfaces bounding the capillary space. It is concluded that the emplacement of the precipitation is made possible by the nearness of these surfaces. In minute glass tubes the precipitation occurs attached as a ring to the walls, without the disc, for the occurrence of which a tube having a diameter greater than a certain critical value is required. This distance is related to the rate of movement of the ions concerned; that is to say, periodic precipitation does not depend for its occurrence on a capillary space, but its fixation depends on the presence of appropriate surfaces. Thus, in a wide enough tube, and in the case, *e.g.*, of cobaltous hydroxide precipitated in gelatin, the precipitate is adsorbed on the glass wall (as a ring) and within or upon the gelatin itself (as a disc). In the absence of a glass (or other) wall only diffusion-shell patterns would arise, as in the classical OSTWALD experiment. It is urged, however, that some surfaces ("nuclei") are required further. That periodicity occurs in a fully fluid medium (water) does not invalidate this conception.

In insisting on the importance of surfaces we approach the position of BRADFORD (1), who believes that adsorption plays an important rôle. The precipitate, he says, furnishes surfaces toward which the surrounding solute

moves, adding to each particle more material. Without pausing here to consider how, according to this author, periodicity consequently arises, we have to say that, the precipitate having once been established, in a particulate condition, the evidence we have seen supports BRADFORD'S ideas. The provision of such particles appears to demand a condition of supersaturation (OSTWALD).

The more surfaces that are provided and the more irregular they are, the more diverse the patterns are which arise, nevertheless, from periodic precipitation. We have been led to see that the heterogeneity of the medium, such as the living cell or body presents, must furnish conditions in which extreme irregularity of pattern would arise from periodic precipitations. We should examine biological material therefore, free, at any rate from prejudice arising out of the contemplation of those beautifully regular patterns (the "LIESEGANG rings") which have engrossed the attention of biologists to the exclusion of many other possibilities.

Differences of temperature have been found to affect the results profoundly. Low temperatures did not favor regularity, or even the obvious occurrence, of periodic precipitation.

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Description of figures

FIG. 1. Trichome (*Saintpaulia* sp.) treated with cobalt sodium hexanitrite, followed, after washing, by ammonium sulphide in 1923 (LLOYD, (7)). The black precipitate has to some extent disappeared (by 1927) leaving only the denser portions.

FIG. 2. Another example, in which only the densest zone of each ring is left. At the level of each ring the cuticle is sharply contracted. (See fig. 3b.)

FIG. 3a. A portion of fig. 1 enlarged.

FIG. 3b. A portion of fig. 2 enlarged.

FIG. 4. Trichomes of *Pelargonium* sp. showing periodic precipitation with irregularity of position, probably the effect of the septa. The precipitate occurs as a fine granulation.

FIG. 5. A trichome of *Pelargonium* sp. with minute tubercles on the surface, underneath which masses of precipitate occur. But the grouping is periodic.

FIG. 6. Normal, and fig. 7 oblique, view of a group of capillary tubes (experiment of 1923) showing periodic precipitation in the form of plates or partial plates. A second system, the nature of which is unknown, is visible as faint bands between the more widely spaced dark bands. Tubes contained gelatin-cobalt sodium hexanitrite; ammonium sulphide as entering reagent.

FIG. 8. Test tube containing 5 per cent. "Difco" gelatin, cobalt reagent 1: 20; entering reagent, ammonium sulphide 1: 5. Gas bubbles, followed by colloidal fracture.

FIG. 9. Similar experiment but with cobalt reagent 1: 5. Distortion of bubbles according to gradient of swelling.

FIG. 10. One half of a capillary tube in which the bubbles form a simulacrum of periodicity. They show distortion due to swelling of the column of gelatin, in amount according to position. The middle point of the tube is above. A blue ring occurs at one end of the 5th visible bubble from the mouth of the tube. Sheet gelatin 5 per cent.—cobalt reagent 1: 16; entering reagent, ammonium sulphide (old) 1: 5. For the form of these bubbles see fig. 12.

FIG. 11. A single bubble in a capillary tube enlarged by spasmodic fracture, simulating periodicity.

FIGS. 12, 13. Gelatin 10 per cent., cobalt reagent 1: 32 in tube, ammonium sulphide entering, 1: 10.

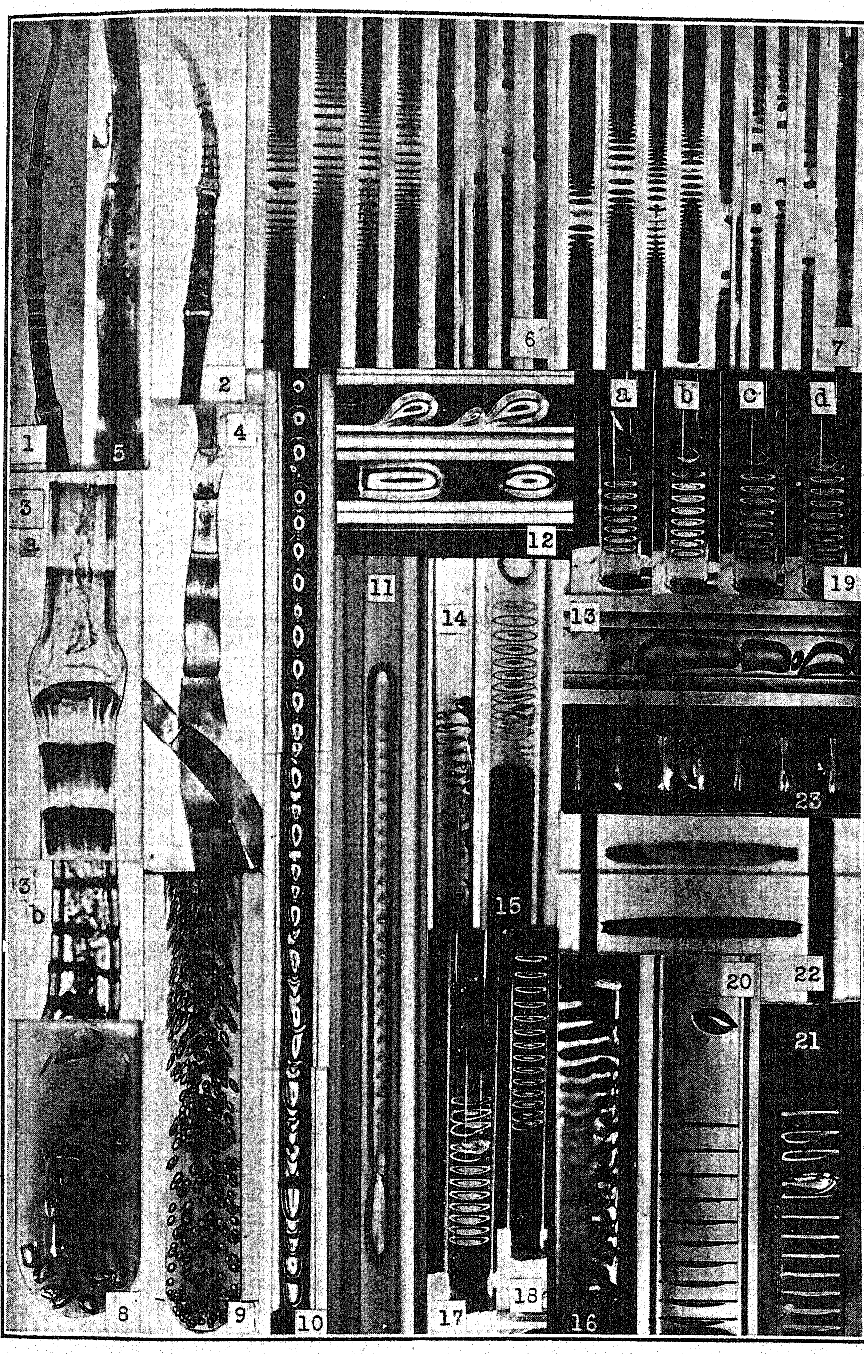
FIG. 12. Bubbles in capillary tubes seen from two points of view. They appear to have originated at the glass wall. With enlargement, displacement due to swelling of the gelatin column.

FIG. 13. Capillary tube with a cobalt ring-disc fractured by strain of gelatin or by passage of a bubble of gas.

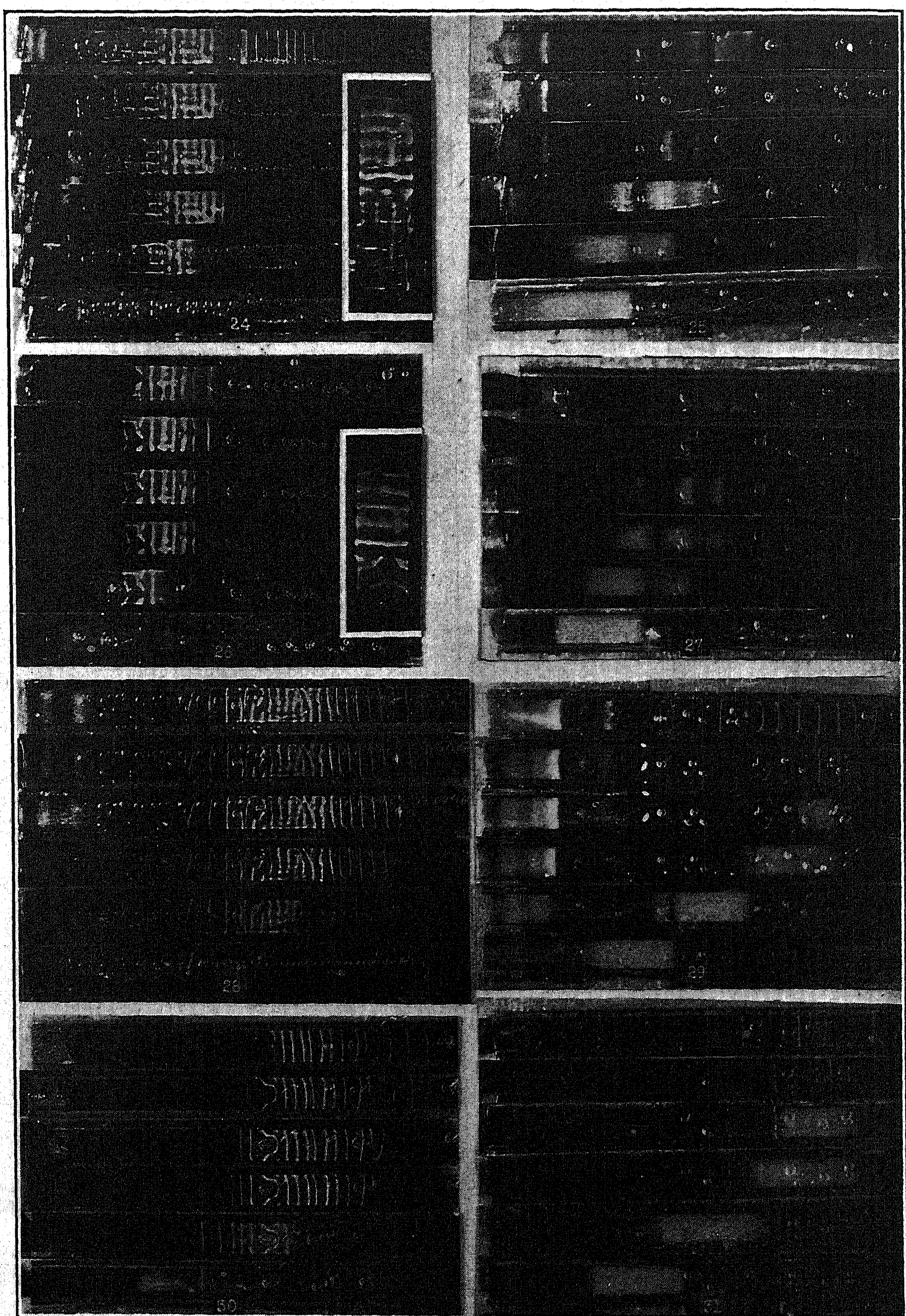
FIGS. 14–23 incl., 32–34 incl. Tubes 2 mm. diam., 10 per cent. "Difco" gelatin; 5 per cent. freshly prepared cobalt reagent; entering reagent, ammonium sulphide 1: 10. Blue cobaltous hydroxide rings followed by black cobalt sulphide rings.

FIGS. 14–15. Periodic precipitations of cobaltous hydroxide in advance of cobalt sulphide. Ring-and-disc structure traceable before periodicity becomes evident.

FIG. 16. Photograph somewhat later of the tube as seen in fig. 14, but by reflected light, bringing out the surface pattern more plainly.



LLOYD AND MORAVEK—PERIODIC PRECIPITATION



LLOYD AND MORAVEK—PERIODIC PRECIPITATION

FIGS. 17–18. Successive photographs of the same preparation by reflected light, viewed obliquely. Reduction and enlargement of discs, and distortion due to bubble.

FIG. 19. Four successive photographs to show (a) the first appearance of a ring, (b) followed by its disc, (c) their increase in density or thickness, and (d) the appearance of the next following ring.

FIG. 20. Displacement of discs due to swelling caused by entering reagent. Transmitted light.

FIG. 21. The same tube somewhat later. Distortion of successive rings in an oblique sense caused by the bubble seen above in fig. 20. Reflected light.

FIG. 22. Cobaltous hydroxide ring just after being completed (above), and after contracting (below).

FIG. 23. Blue cobaltous hydroxide rings covered by a *white* precipitate. Photographed through a blue absorbing filter.

FIGS. 24–31. Eight tubes containing 20 per cent. "Difco" gelatin, 5 per cent. cobalt reagent. The tubes of each sextette are regarded as numbered from 1 to 6 from the bottom, in each case. Tubes nos. 1, after 25 hours; nos. 2, after 52 hours; nos. 3, after 100 hours; nos. 4, after 114 hours; nos. 5, after 148 hours; nos. 6, after 244 hours.

FIGS. 24–25. Entering reagent ammonia 1: 20.

FIGS. 26–27. Entering reagent, ammonium sulphide 1: 20.

FIGS. 28–29. Entering reagent, ammonia 1: 5.

FIGS. 30–31. Entering reagent, ammonium sulphide 1: 5.

FIGS. 24, 26, 28, 30. At room temperature throughout period of observation.

FIGS. 25, 27, 29, 31. In cold (3° C.) (tubes 1–3), afterwards (tubes 4–6) at room temperature.

FIGS. 24, 26. Insets, enlargements of precipitate in tubes nos. 3 of the sextettes of these figures.

FIGS. 32–34. Showing secondary changes which take place in the cobalt sulphide rings. The blue rings (seen as white bands in advance of the black column) are persistent and become blackened. Compare with figs. 64, 69, which are these rings set free.

FIG. 35. Test tube with very crowded sulphide rings. *Cf.* fig. 38, which is a thin longitudinal section after removal from tube by breaking away the glass. 10 per cent. "Difco" gelatin; cobalt reagent 1: 10. Entering reagent ammonium sulphide 1: 10.

FIG. 36. Cobalt sulphide rings in a capillary tube, pushed away from the mouth of the tube. Before the swelling of the gelatin column they were indistinguishable, and are yet, where swelling has not intervened.

FIG. 37. Rings (true) and plates of cobalt hydroxide in advance of cobalt sulphide. The plates are formed by filling up the space by centripetal spreading of the ring in capillary tubes.

FIG. 38. See under fig. 35.

FIG. 39. In advance of black rings-discs is a brown zone free of precipitate and free from blue rings which have disappeared by dissolution. Entering reagent at first ammonia, for which, later, after the blue system was complete, ammonium sulphide was substituted.

FIG. 40. Test tube photographed from three positions to avoid parallax as far as possible. Cobaltous hydroxide.

FIG. 41. A series of tubes of various sizes containing a gelatin-ammonium sulphide mixture. Entering reagent, air. Lowermost tube, control, its upper end (on the right) hermetically sealed.

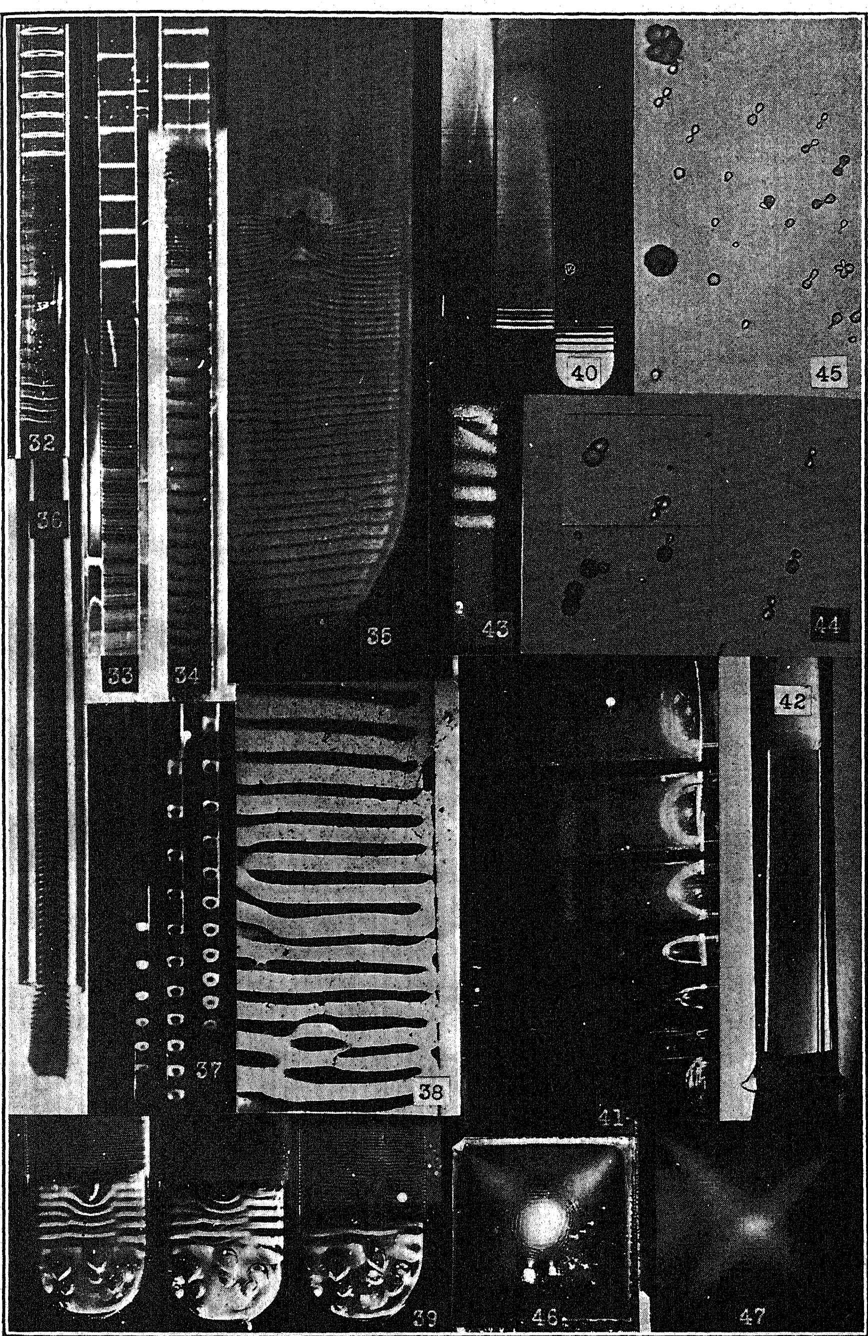
FIGS. 42–43. Tube filled with cobalt reagent 0.5 per cent., gelatin 20 per cent. mixture. Entering reagent: ammonia diluted 1: 50 (fig. 42), followed by ammonia diluted 1: 10, after which microcrystalline sulphur precipitated out in rings (fig. 43).

FIG. 44. Dumb-bell shaped microcrystalline sulphur obtained from rings of the tubes shown in fig. 41.

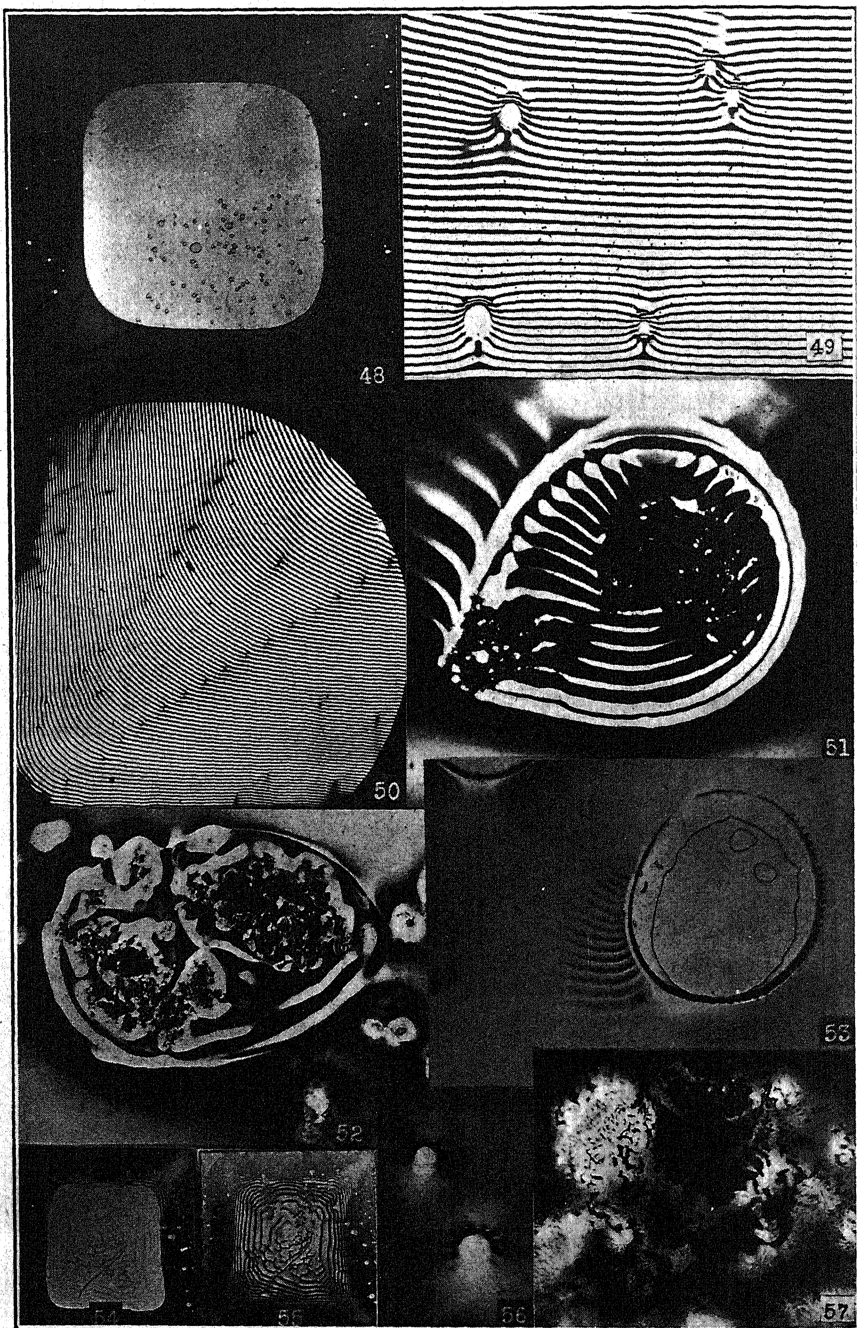
FIG. 45. Microcrystalline sulphur from the tube shown in fig. 43.

FIG. 46. Diffusion accompanied by periodic precipitation between glass plates inclosing gelatin 10 per cent. ("Difco") + 5 per cent. cobalt reagent. Entering agent, ammonium sulphide 1: 50.

FIG. 47. "Cross" diffusion pattern between glass plates, without visible periodicity. Between plates: "Gold label" gelatin 10 per cent. + cobalt 1: 32; entering reagent, ammonium sulphide 1: 10.



LLOYD AND MORAVEK—PERIODIC PRECIPITATION



LLOYD AND MORAVEK—PERIODIC PRECIPITATION

FIG. 48. Between glass plates: gelatin 10 per cent. ("Difco") + cobalt reagent 1: 10; entering reagent ammonium sulphide 1: 10 + ammonia.

FIG. 49. Between glass plates: gelatin 10 per cent. ("Difco") + cobalt 1: 10 + acetic acid; entering reagent, ammonium sulphide 1: 10.

FIG. 50. Detail from corner of fig. 48.

FIGS. 51, 52. Between glass plates: gelatin 10 per cent. ("Difco") + cobalt reagent 1: 10 with gypsum mixed in; entering reagent, ammonium sulphide 1: 5.

FIG. 53. Between glass plates: gelatin 5 per cent. ("Difco") + cobalt reagent 1: 5; entering reagent, ammonium sulphide, 1: 5.

FIG. 54. Between glass plates: gelatin 10 per cent. ("Difco") + cobalt reagent 5 per cent.; allowed to set 24 hours at 13° C. Entering reagent freshly prepared 1: 10. Cobaltous hydroxide rings visible beyond advancing margin of cobalt sulphide. The same preparation photographed later is seen in the next figure.

FIG. 55. Cobalt sulphide rings now accentuating the pattern formed by the cobaltous hydroxide rings seen in fig. 54.

FIG. 56. Set up as in fig. 53, but a thicker layer of the gelatin.

FIG. 57. Cobalt sulphide rings deposited at a gelatin-oil interface secondarily changed by evaporation of the metastable precipitate accompanied by the growth of minute, feathery sulphur crystals. Set up as for fig. 51, but oil droplets instead of gypsum.

FIG. 58. Tube = 6 mm. diameter containing gelatin 20 per cent. ("Difco"), cobalt reagent 1: 20 with a glass rod running approximately along the axis. Entering reagent, ammonia 1: 5.

FIG. 59. The same, but entering reagent, ammonium sulphide 1: 10.

FIG. 60. Glass rod after removal from the tube (fig. 59), on the left. The rod from tube, fig. 58, on the right.

FIG. 61. Portion of fig. 60 (left hand figure), enlarged.

FIG. 62. Same as fig. 58, but glass fragments instead of a rod.

FIG. 63. Detail from fig. 62 highly magnified. Precipitate is seen attached to glass fragments.

FIG. 64. Rings and discs of both cobaltous hydroxide blackened with cobalt sulphide (small ones), and cobalt sulphide rings and discs (larger ones); the shorter arrow points to the latter.

FIG. 65. Capillary tubes showing rings (strictly understood) with increasing width. Displacement of one ring due to a bubble.

FIGS. 66–68. Longitudinal sections of the gelatin column from test tubes containing cobaltous hydroxide precipitate, showing ring-and-disc structure.

FIG. 69. Portion of gelatin column from capillary tube (fig. 32) after being expelled therefrom by swelling. On the right: separated rings and part of a disc.

FIG. 70. Transverse section of gelatin column of test tube, fig. 40, near bottom of tube where the rings are separate, showing ring-and-disc structure. The disc is not completely circular, and has a pore in it, besides being of uneven thickness. The incompleteness of the ring is due to difficulty in complete removal from the glass wall.

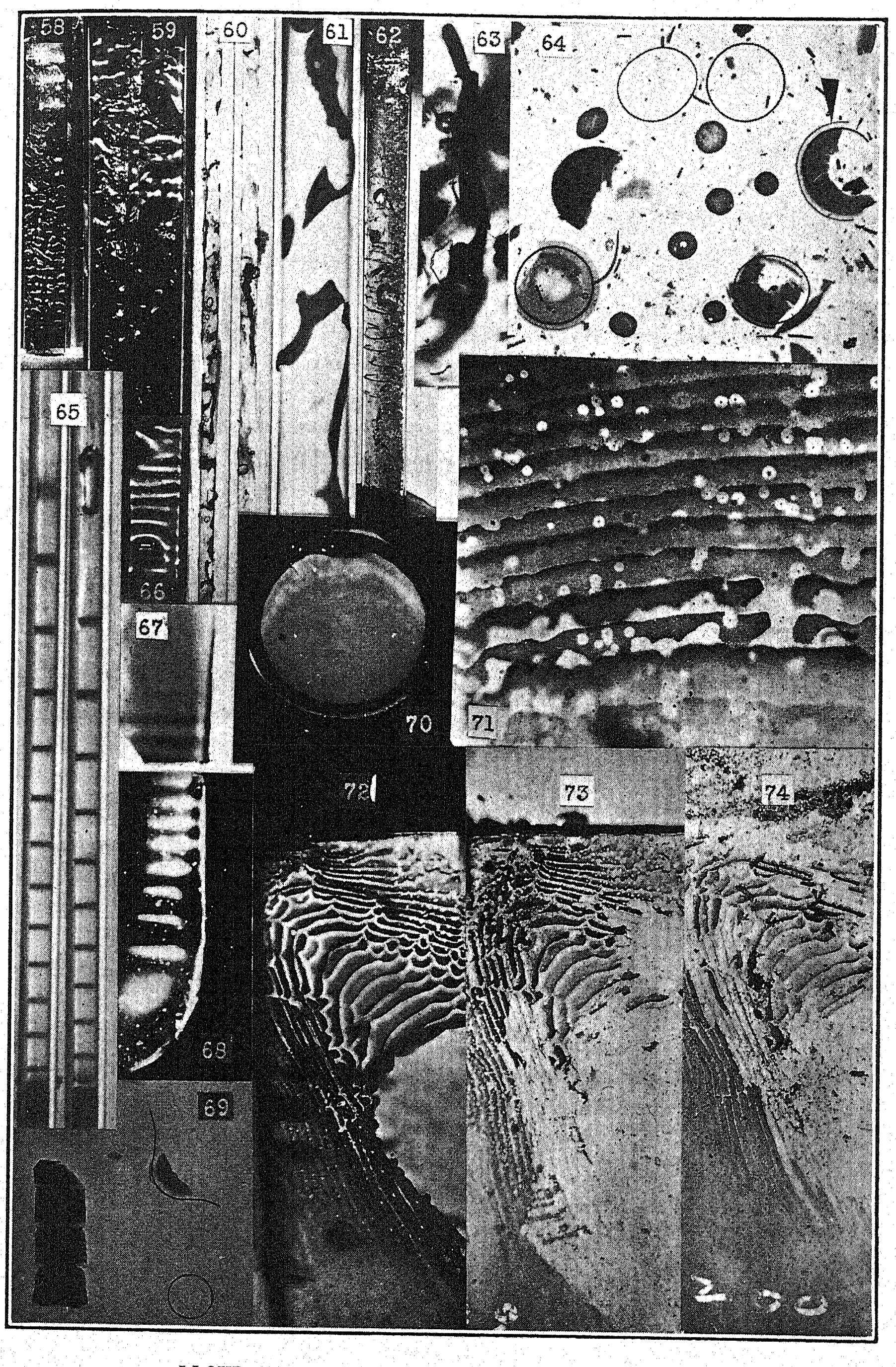
FIGS. 71–74. Periodic precipitation between glass plates from fluid cobalt reagent alone (without gelatin), with equal parts of ammonium sulphide and glycerine as entering reagent.

FIG. 71. Similar deposition forms of periodic precipitation between glass plates slightly displaced. The alternate rings in sharp focus are on the upper glass face; those out of focus, on the lower.

FIG. 72. Another precipitation, viewing the whole precipitate.

FIG. 73. That on the upper glass plate of fig. 72.

FIG. 74. That on the lower glass plate of fig. 72.



LLOYD AND MORAVEK—PERIODIC PRECIPITATION

CHANGES IN THE BUFFER SYSTEM OF THE WHEAT PLANT DURING ITS DEVELOPMENT

ANNIE M. HURD-KARRER

(WITH TWELVE FIGURES)

Introduction

In an earlier paper (5) it was reported that progressive changes occur in the acidity of the wheat plant during its seedling and maturation stages. Subsequent studies involving the complete titration curve have given a more comprehensive idea of the changes which take place in the titratable compounds of the juice. The results show a remarkably ordered relation between the buffer capacity of the juice, its hydrogen-ion and ''titratable-acid'' concentration, and the stage of development of the plants.

Methods

The varieties, White Odessa, a winter common wheat, and Jenkin, a spring club wheat, were grown for this investigation in a greenhouse at the Arlington Experiment Farm. At predetermined intervals, some of the plants were cut and the juice expressed and titrated electrometrically as rapidly as possible, according to a uniform procedure. Ten cubic centimeters of the undiluted juice were taken for each titration. As soon as the hydrogen electrode had come to equilibrium with the juice, *i.e.*, after about 20 minutes, the sample was titrated with N/20 sodium hydroxide. Immediately following the alkali titration a second 10-cc. sample of the juice was similarly titrated with N/20 sulphuric acid. The electrode was replatinized for each titration. Details of procedure are given in an earlier paper (**4**).

The potential differences measured after the addition of each successive cubic centimeter of alkali or of acid to the juice were translated into pH values and plotted against the quantities of alkali or acid producing them. The ''blank'' corrections (**11**) for these values, *i.e.*, the quantities of alkali required to bring equivalent volumes of water alone to the same pH values, were so small over the range of these titrations that they could be ignored. Only actual observations are plotted in the graphs.

Results

1. CHANGES IN THE FORM OF THE TITRATION CURVE DURING THE GROWTH PERIOD

For the experiments of 1926 the seed was sown in January, part of it being broadcasted for the work on very young seedlings, and the rest spaced

in rows. The surface soil was removed to a depth of about four inches and replaced by soil carefully sifted and mixed to insure uniformity. The temperature[1] and soil moisture were kept as favorable for the growth of wheat as possible. It was necessary to maintain fairly uniform moisture conditions in order to obtain comparable titrations.

Material for the measurements was collected about every other day for the first two weeks after the emergence of the seedlings, and weekly or

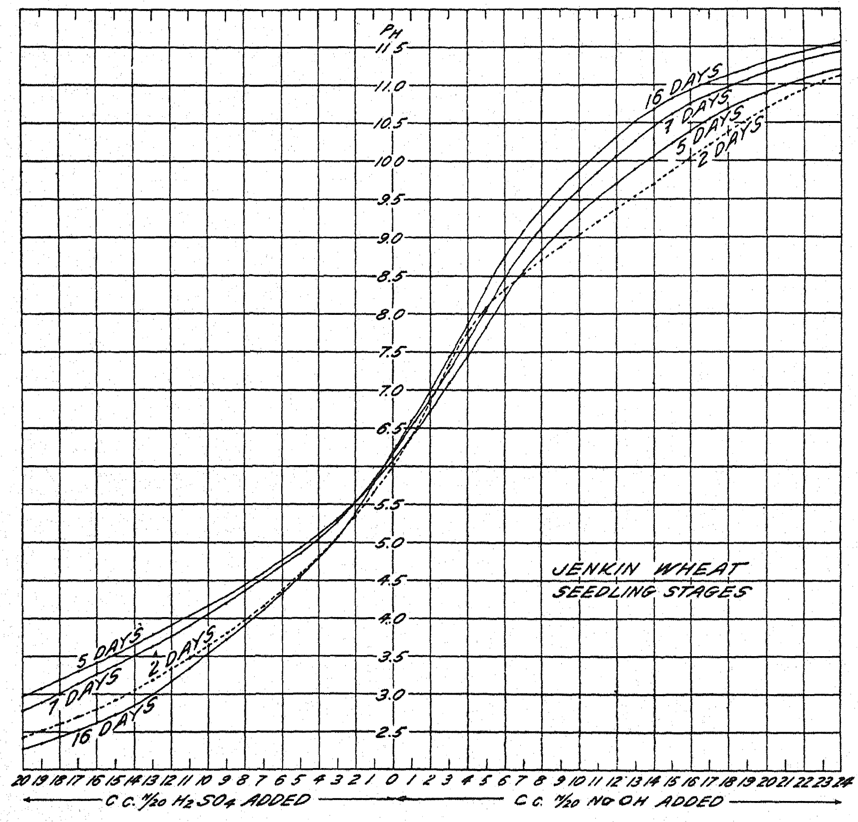


FIG. 1. Titration curves showing changes in the composition of juice of wheat (Jenkin) during the seedling stage.

[1] The air temperature was kept close to 16° C. during January, February, and March, falling to about 13° at night, and occasionally rising above 20° on warm afternoons. The soil temperature remained near 15° during this period. In April and May the air temperature was higher, frequently rising to 35° in the afternoon, and falling to 16° at night. The soil temperature fluctuated daily between 16° and 21°. During June, while the plants were maturing, the air temperature was frequently above 40°, with the soil temperature ranging from about 18° on the coolest days to 26° on the warmest days.

biweekly thereafter until the plants matured. The plants were cut just above the ground, the dead or dying lower leaves and culms of old plants being discarded. The heads were removed from plants in the booting stage and later. Age was reckoned from the date of emergence.

In figs. 1 and 2 are plotted the titration values obtained during the seedling stage. The curves show that a progressive change in the buffer

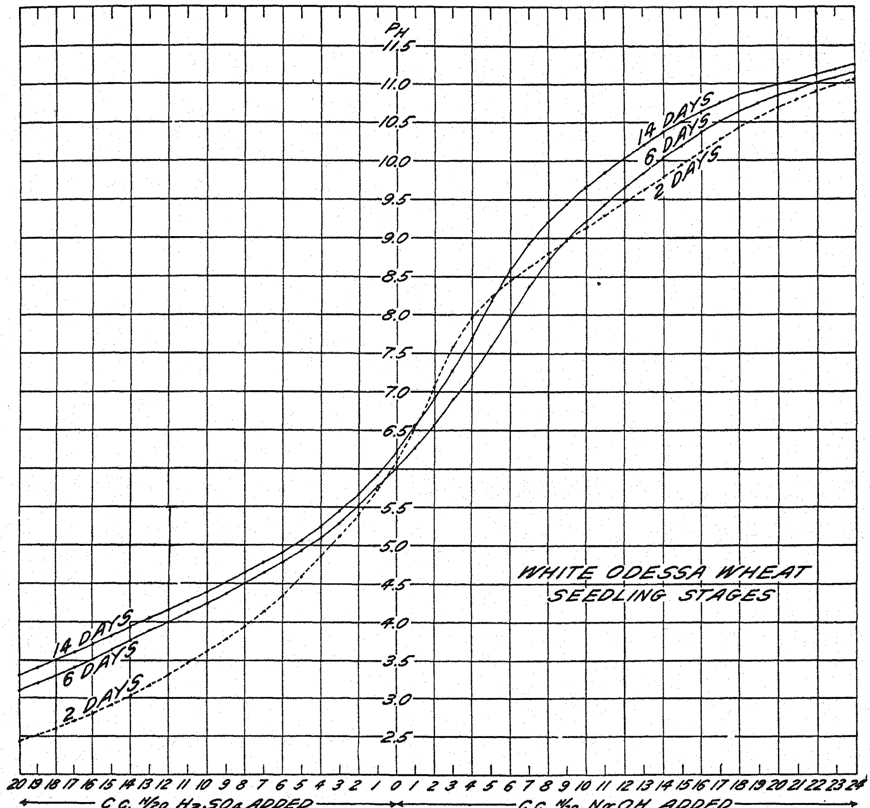


FIG. 2. Titration curves showing changes in the composition of juice of wheat (White Odessa) during the seedling stage.

system occurs during the first two weeks after emergence. When the plants are not over one inch high, with the first leaf not yet unfolded, the curve is very much flattened between about pH 8.5 and 10.5.[2] In fact, at a still earlier stage, when the plants are just showing above the ground, a curve may be obtained which is slightly depressed between these limits, as shown

[2] Broken lines are used in drawing the curves for these young seedlings wherever it is desirable to differentiate them more clearly from the curves for the older seedlings.

in fig. 3. Then in a few days this section of the curve becomes distinctly convex. The point of inflection changes from about pH 7.5 to a point near 8.0. The degree of convexity increases regularly through the 1-leaf and 2-leaf stages, or until about two weeks after emergence. Over the acid range, the curves become less and less steep as the plants develop. Thus during the seedling stage the buffer capacity of the juice is increasing in the

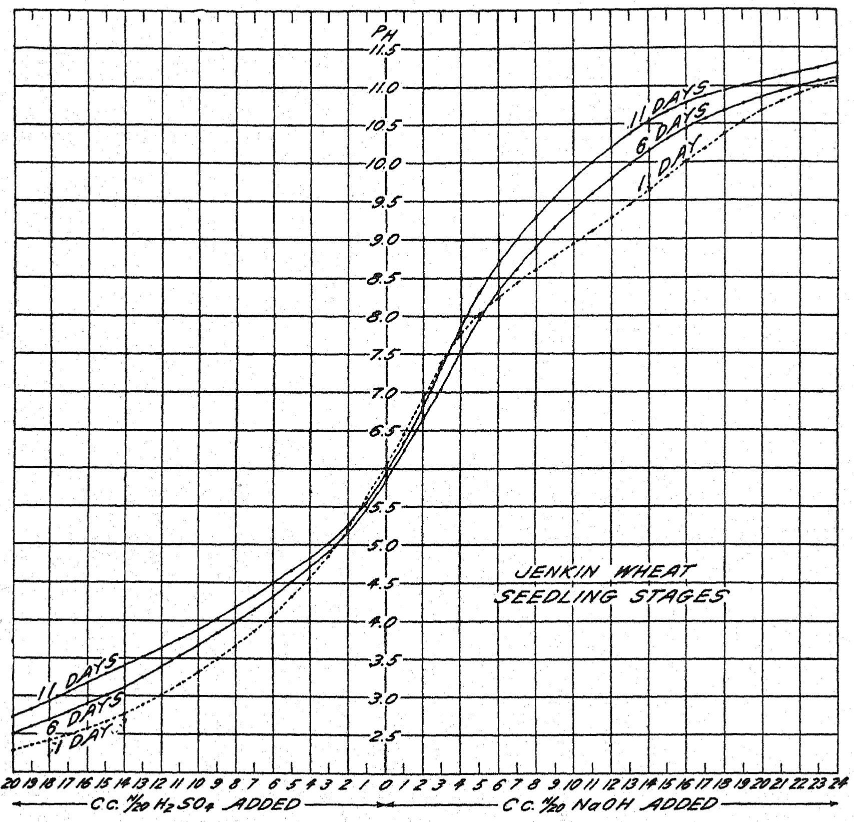


FIG. 3. Titration curves showing changes in the composition of juice of wheat (Jenkin) during the seedling stage.

acid range of the titrations while it is decreasing in the alkaline range.

The sequence of changes shown in figs. 1 and 2 is essentially the same in seedlings grown under quite different environmental conditions. In figs. 3 and 4 are shown corresponding sets of curves obtained in a preliminary experiment in 1925, with plants grown from seed sown three months later in the season (April) in a different greenhouse.

It appears from these curves that during the period of nutritional adjustment, between the emergent and the late-seedling stages, there occurs a change in the chemical composition of the juice which is reflected in the changing form of the titration curve. Curves for etiolated seedlings were found to resemble those for the emergent seedlings, being similarly flattened, and usually distinctly depressed, between pH 8.5 and 10.5. It is concluded, therefore, that assimilatory processes are not yet fully established in the

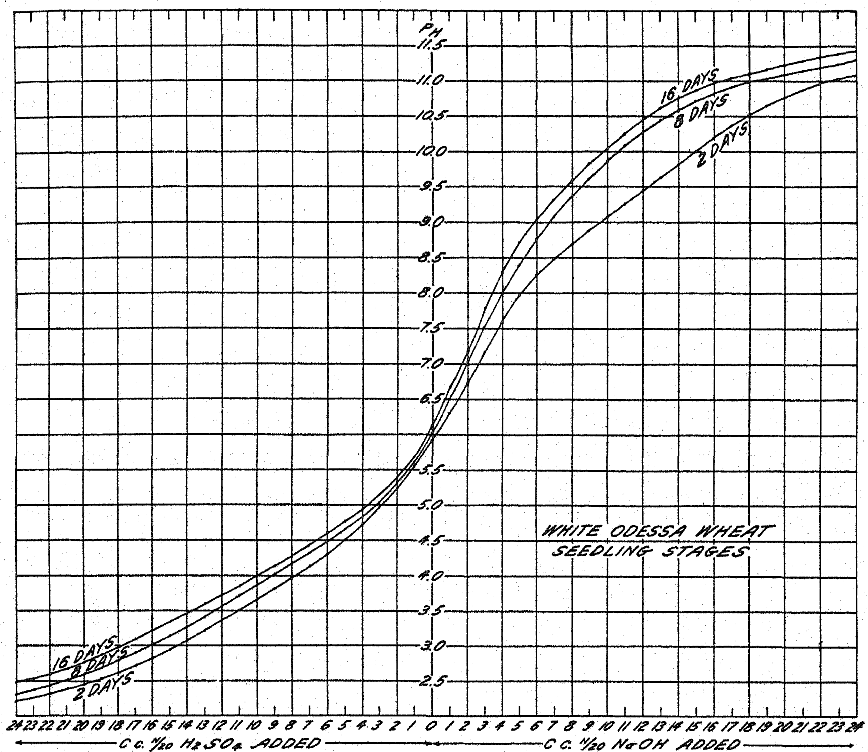


FIG. 4. Titration curves showing changes in the composition of juice of wheat (White Odessa) during the seedling stage.

very young greenhouse seedlings, even after they become green. The subsequent curves, increasingly convex between pH 8.5 and 10.5, evidently show the transition from the stage of complete dependence on the endosperm, to the stage of full photosynthetic activity. The curve of maximum convexity then indicates the stage when the constituents of the juice have reached an equilibrium concentration. This curve may be considered as marking the end of the seedling stage from a physiological standpoint.

Throughout the tillering stage and during the greater part of the shooting stage there are only small fluctuations in the position of the curve, probably caused by unavoidable variations in environmental conditions. The curves for this period lie so close to those of the late seedling stage that they can not be easily distinguished when plotted on the same chart. Therefore they are not included in the figures.

The first significant departure from this type of curve was obtained toward the end of the shooting stage. The juice became more highly buf-

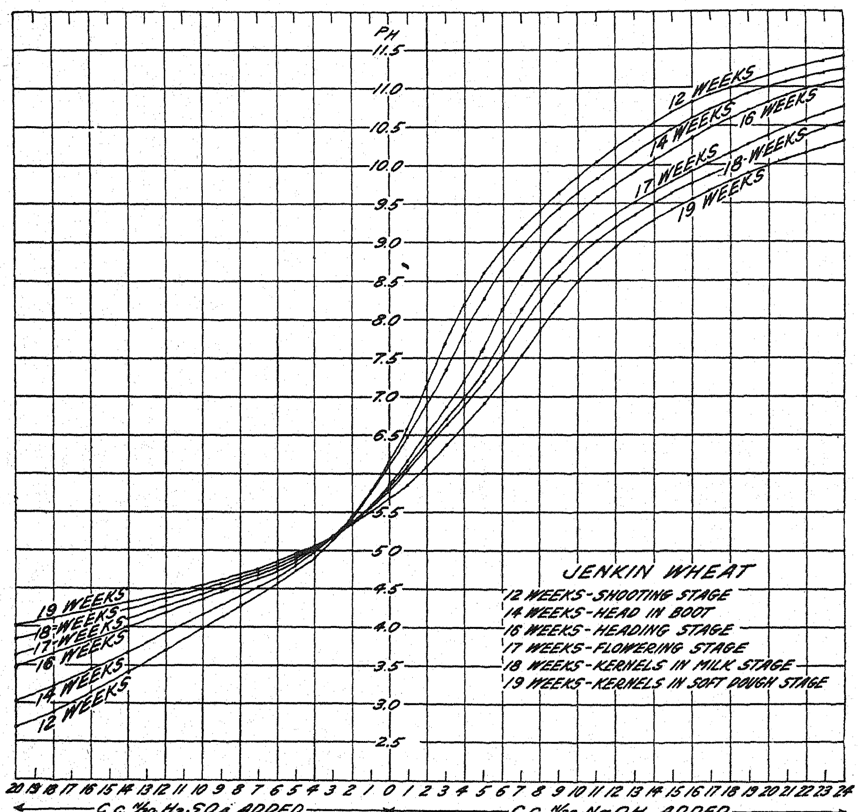


FIG. 5. Titration curves for juice of wheat (Jenkin) during the maturation period.

fered against both alkali and acid. This change marks the beginning of a period which continues throughout the stages of heading, flowering, and kernel development, during which there is a progressive increase in the buffer action of the juice (figs. 5 and 6[3]).

[3] Curves obtained for plants of White Odessa 13 to 16 weeks old were omitted from fig. 6 because they lay too close to each other, and to those obtained at 12 and 17 weeks, to be distinguishable. A curve representative of plants 20 weeks old also was omitted, as it was practically the same as the curve for 19 weeks.

The successive changes in the titration curve of White Odessa were less regular in the alkaline range than were those of Jenkin, although the same correlation of age with degree of buffer action was evident. White Odessa grew less normally and matured more slowly under greenhouse conditions than Jenkin, requiring 22 weeks to reach the soft-dough stage of the kernels as compared to the 19 weeks required by Jenkin to reach the same stage.

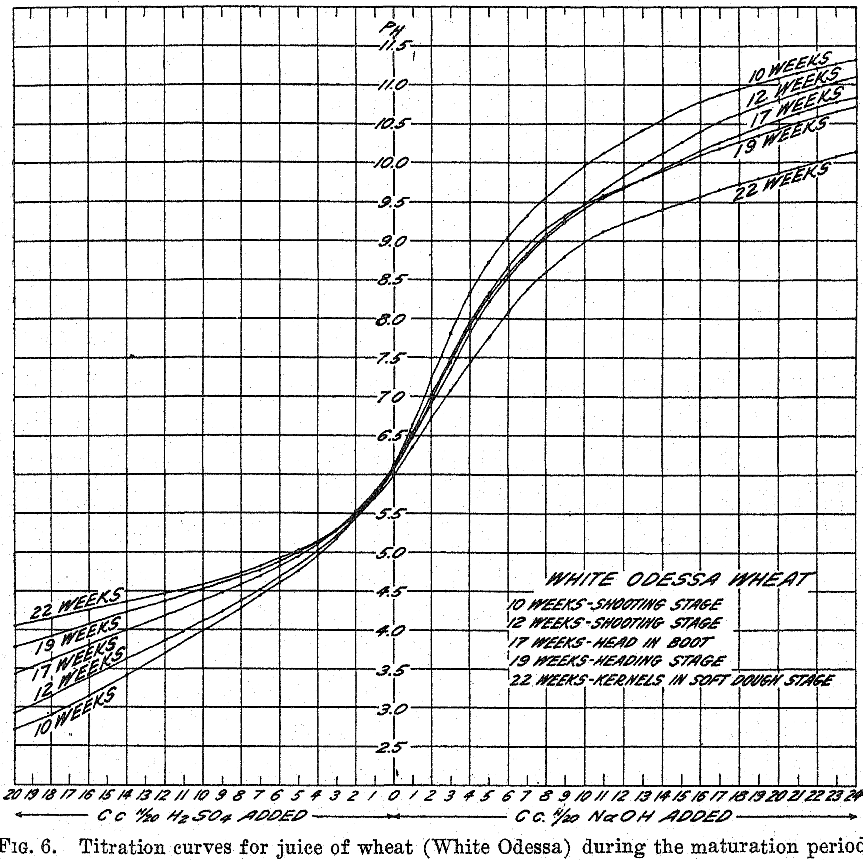


FIG. 6. Titration curves for juice of wheat (White Odessa) during the maturation period.

Also, White Odessa became slightly infected with mildew, which may have contributed to the greater irregularity of the change in the curve.

Figures 5 and 6 show that the curves for both Jenkin and White Odessa intersect between pH 5.3 and 6.0; that each curve passes through a point of inflection near pH 8.0; and that the slopes of the curves over both the acid and alkaline ranges decrease with increasing age of the plants.

The interrelationships of these curves suggest an analogy with the intersecting curves obtained on titrating different concentrations of a simple buffer solution such as the phthalate, phosphate, or standard acetate mixture. In the region of their intersection the curves resemble also those of SØRENSEN (**10**) which represent titrations of different concentrations of egg albumin. Thus it appears that the differences in buffer capacity shown from week to week during this period were due to progressive changes in the concentration of the juice as the plants ripened and dried.

2. The change in concentration of the juice during the maturation period

It is a matter of common observation that during the maturation period the tissues of the wheat plant become less and less succulent until, by the time the kernels are in the soft-dough stage, comparatively little juice can be expressed. The changes in the "titratable acid" values observed during this period were found (**5**) to be correlated with the rate of drying of the tissues. These observations as well as the characteristics of the curves themselves made it appear probable that the changes in buffer action were the result of increasing concentration of the juice and not due to metabolic changes.

In order to determine whether the concentration of the juice changes during this period, series of specific gravity measurements were made between the shooting and the soft-dough stages. Plants of both Jenkin and White Odessa were grown in 1927 in the same greenhouse, and under as nearly as possible the same conditions, as those maintained for the experiments of 1926. At intervals during the maturation period, juice was expressed and titrated, and its specific gravity determined by means of a small pycnometer. The juice was clarified for the specific gravity measurements by heating it carefully to 60° C. and filtering. The possibility of appreciable error resulting from the precipitation of solutes by this temperature was thought more remote than the danger of changing the concentration by filtering through paper pulp. The data are given in tables I and II and in fig. 7.

These data show that the "titratable acid" in the juice increases during the maturation period, and that the specific gravity increases, in general, at a corresponding rate. That the rate of increase in "titratable acid" is determined largely by the increase in density is shown by the fact that the ratio of the approximate weight of solids in a given volume of juice (the specific gravity measurement minus one) to the titratable-acid measurement is so nearly the same at each stage of development. It is interesting to note

that the buffer system functioned so effectively during this period that the hydrogen-ion concentration remained fairly constant up to the final measurement.

TABLE I

THE pH, "TITRATABLE ACID," AND SPECIFIC GRAVITY VALUES FOR JUICE OF JENKIN WHEAT DURING THE MATURATION PERIOD (EXPERIMENTS OF 1927)

AGE IN WEEKS	STAGE OF DEVELOPMENT	pH	"TITRATABLE ACID," (CC. N/20 NaOH REQUIRED TO REACH pH 8.3) A	SPECIFIC GRAVITY B	$\frac{B-1}{A}$
13	shooting	5.77	6.2	1.0269	0.0043
14	shooting	5.77	6.4	1.0219	0.0034
15	heads in boot	5.74	7.0	1.0334	0.0048
15½	heading	5.78	7.2	1.0318	0.0044
16	heading	5.74	7.4	1.0312	0.0042
17	flowering	5.77	10.4	1.0461	0.0044
18	kernels in milk stage	5.76	11.9	1.0576	0.0048
19	kernels in soft-dough stage	5.71	10.0	1.0491	0.0049
20	kernels in soft-dough stage	5.66	11.6	1.0557	0.0048

Specific gravity measurements thus show that the change in form of the titration curve during the maturation period is correlated with increasing concentration of the juice. Consequently the first departure from the type

TABLE II

THE pH, "TITRATABLE ACID," AND SPECIFIC GRAVITY VALUES FOR JUICE OF WHITE ODESSA WHEAT DURING THE MATURATION PERIOD (EXPERIMENTS OF 1927)

AGE IN WEEKS	STAGE OF DEVELOPMENT	pH	"TITRATABLE ACID," (CC. N/20 NaOH REQUIRED TO REACH pH 8.3) A	SPECIFIC GRAVITY B	$\frac{B-1}{A}$
12	shooting	5.74	7.8	1.0249	0.0032
13	shooting	5.81	7.9	1.0260	0.0033
15	shooting	5.74	8.0	1.0287	0.0036
16	shooting	5.81	8.4	1.0336	0.0040
17	heading	5.73	11.0	1.0455	0.0041
17	flowering	5.85	10.1	1.0470	0.0047
18	late flowering	5.77	13.2	1.0481	0.0036
19	kernels in milk stage	5.73	14.0	1.0566	0.0040
20	kernels in soft-dough stage	5.83	14.7	1.0690	0.0047
21	kernels in soft-dough stage	5.55	13.1	1.0594	0.0045

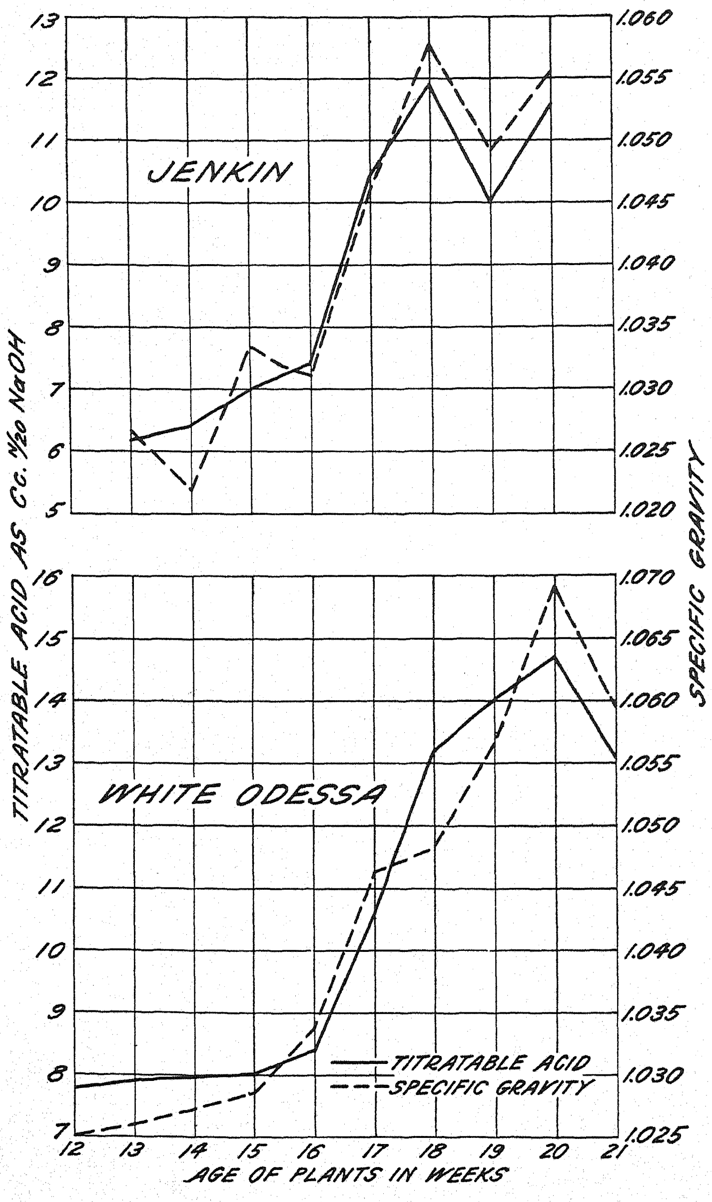


FIG. 7. Correlation between the titratable acid and specific gravity of juice of Jenkin and White Odessa wheat during the maturation period.

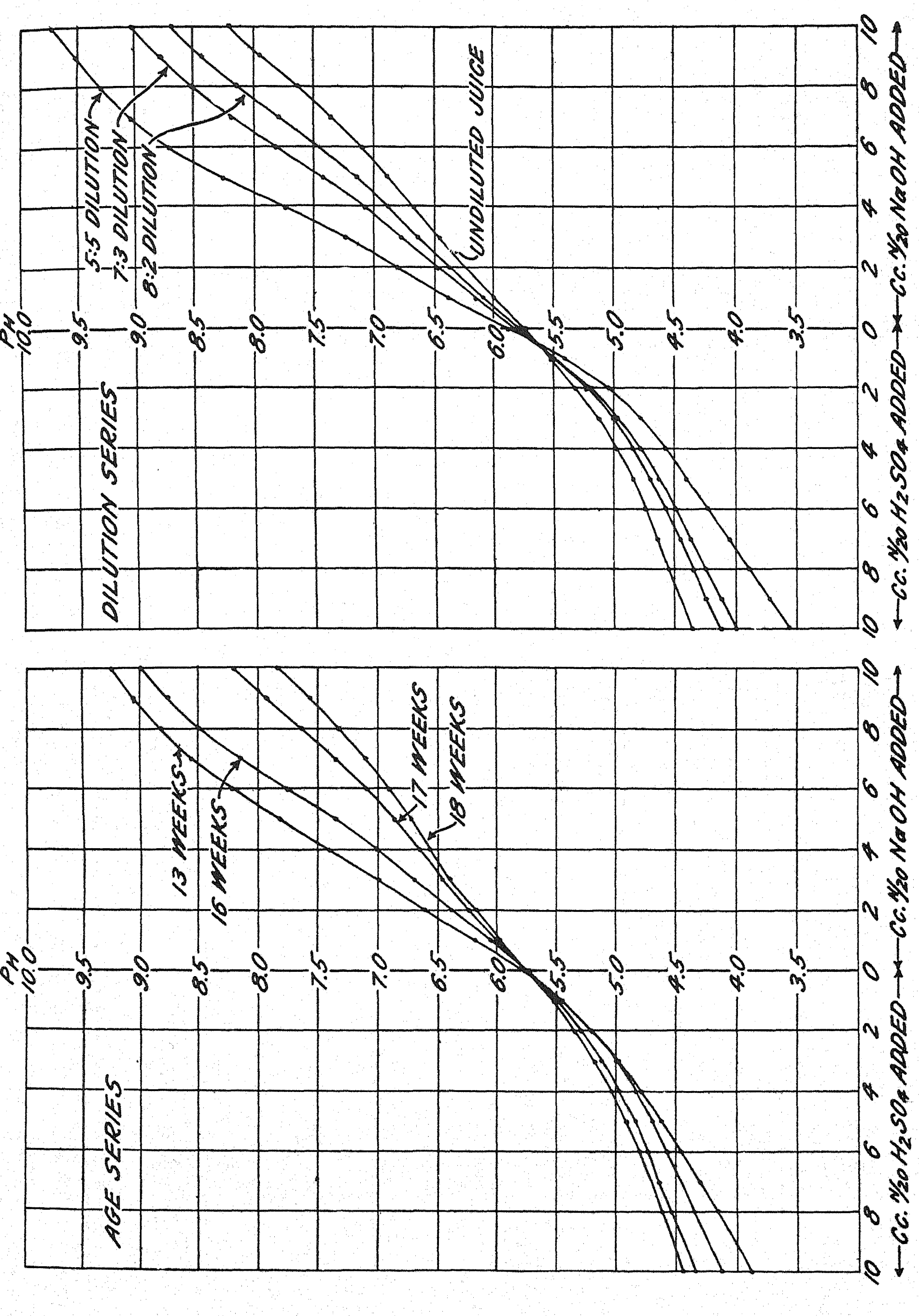


FIG. 8. Titration curves for juice of wheat (Jenkin) in different stages of development compared with artificially diluted juice.

of curve obtained at the end of the seedling stage, and also throughout the subsequent stages of tillering and shooting, marks the beginning of the maturation period.

If change in concentration is solely responsible for the differences in the curve at the successive stages of development, then artificial dilutions of juice from plants in a late stage of development should give curves similar to those obtained for the undiluted juice at earlier stages. Titrations of a series of dilutions of juice from 17-weeks-old plants is shown in fig. 8 together with four curves representing the titrations of undiluted juice from the plants at different stages of development. The resemblance between the two groups of curves is sufficiently close to indicate that change in concentration alone accounts for the change in the titration values observed during the maturation period.

3. Effects of the increasing concentration of the juice during the maturation period on its buffer values

The slopes of a titration curve show graphically the buffer capacity of a solution at each stage of titration. Over any given reaction range, the ratio of the pH increment, ΔpH, to the corresponding increment of alkali (or acid), ΔB, gives a measure of the buffer capacity. This ratio is more convenient than the ratio $\frac{dB}{dpH}$ suggested by Van Slyke (**12**), since ΔB is always equal to one, and it is just as useful for the purpose of the present investigation. It is defined as the change in pH produced by the addition of each cubic centimeter of N/20 NaOH or of N/20 H_2SO_4 to the juice during the titration. It is essentially the reciprocal of the buffer unit $\frac{\Delta(S-S_0)}{\Delta p}$ suggested by Koppel and Spiro (**6**), since the corrections for the effects of water alone (S_0) over the reaction ranges involved are practically zero. Inclusion of a correction for the dilution error is immaterial for values between pH 3.0 and pH 11.0 (**7**).

In fig. 9 are plotted the values of $\frac{\Delta pH,}{\Delta B}$ with the increments in alkali (or acid) as abscissae, for the series of titrations made in 1926 for Jenkin wheat during its maturation period. Since the buffer action at any given point in the titration varies inversely with the change in pH produced by the corresponding increment of alkali or acid, the highest point on each curve marks the point of minimum buffer action. It should be noted that the converse of this statement, namely, that the lowest point on each curve marks the point of maximum buffer action, is not true according to the usual meaning of the term buffer action, which refers to the action of the

solutes only. Over the regions of their flattened ends, where the reaction of the solution is changing most slowly, the titration curves approach asymptotically a line through the pH value of the reagent being added and parallel to the axis of abscissae. VAN SLYKE (12) refers to the slow rate of change of reaction in these regions as due to the buffer effect of water.

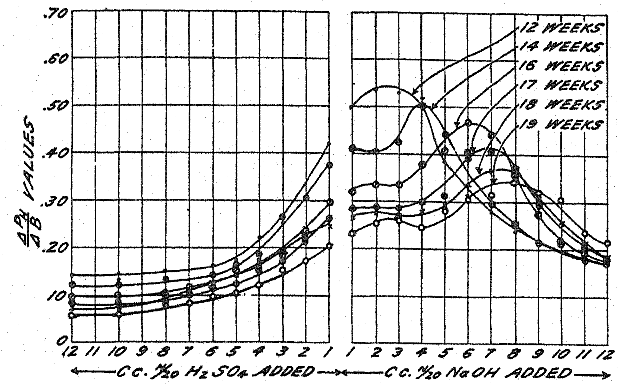


FIG. 9. Graphs showing change in $\frac{\Delta \text{pH}}{\Delta \text{B}}$ values for juice of wheat (Jenkin) during the maturation period.

Minimum buffer action occurs at a different point on each curve in fig. 9, but is found, by reference to the corresponding pH values (fig. 5), to be always near pH 8.0.[4] This constancy is evidence that no radical change takes place in the composition of the juice during the maturation period. The fact that the point of minimum buffer action occurs farther along the axis of abscissae in each successive titration is evidence of the increasing concentration of the juice as the plants ripen and dry.

Artificially diluting the juice of plants in late stages of development causes the buffer values to approximate those of undiluted juices of plants in earlier stages of development. In fig. 10-A are plotted the values of $\frac{\Delta \text{pH},}{\Delta \text{B}}$, with the increments of alkali (and acid) as abscissae, for the titrations shown in fig. 8 for diluted juice from nearly mature plants. In fig. 10-B are shown similar curves for the titrations of the undiluted juice of the plants when they were at different stages of development. The curves

[4] This point of minimum buffer action does not indicate exhaustion of the titratable materials in the juice, for the "blank" corrections show that it takes less than 0.1 cc. of the alkali to bring equivalent volumes of water alone to this reaction. In fact, the "blank" corrections are negligible over the entire range of these curves, showing that the titratable materials are far from exhausted at pH 8.0.

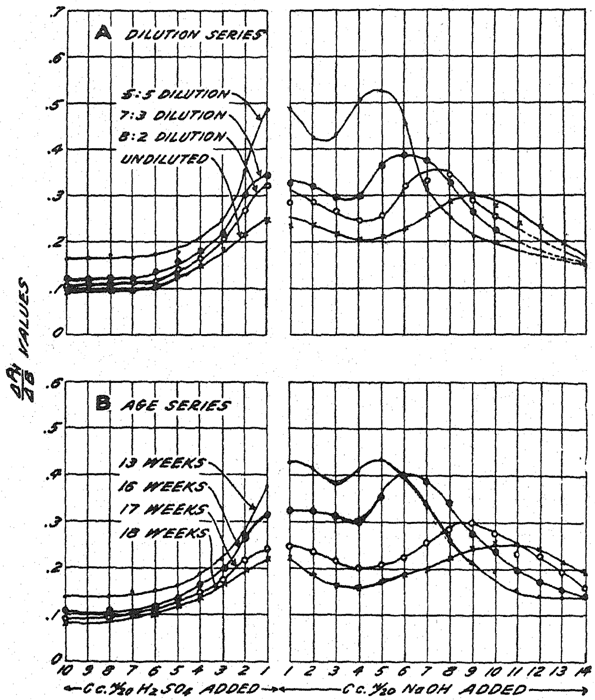


FIG. 10. $\frac{\Delta \, pH}{\Delta \, B}$ values for juice of wheat (Jenkin) in different stages of development and the corresponding values for a series of artificial dilutions of juice.

show that the buffer action varies with age in the natural juices as with concentration in artificially diluted juices.

Comparison of the undiluted juice of plants of different ages with the artificial dilutions is facilitated by the summary in table III. It is noteworthy that the hydrogen-ion concentration of the natural juice is practically the same (pH 5.7–5.8) at each stage of development, but is decreased appreciably by artificial dilution.

4. Preliminary studies on the nature of the titratable constituents in wheat juice

When added to a complex mixture like wheat juice, alkali and acid undoubtedly react with several types of compounds, all of which, if present in sufficient quantity, affect the course of the titration and the so-called "titratable acidity" values. These compounds include organic acids and bases with their salts, and organic and inorganic ampholytes. Little is known concerning the identity and the relative quantities of these compounds occurring in wheat juice.

The buffer systems investigated by HEMPEL (3), working on succulent plants, and YOUDEN and DENNY (13), on extracts of potato tubers, consist mainly of organic acids and their salts. LEUTHARDT (7) also has shown that the titratable compounds in fruits, including apples, grapes, lemons, and tomatoes, are largely organic acids and their salts. He found that sugars are responsible for considerable buffer action at pH values above 9.0.

TABLE III

COMPARISON OF WHEAT JUICE (JENKIN) FROM PLANTS OF DIFFERENT AGES BETWEEN THE SHOOTING AND SOFT-DOUGH STAGES WITH ARTIFICIAL DILUTIONS OF JUICE FROM PLANTS IN THE FLOWERING STAGE

AGE OF PLANTS IN WEEKS	SPECIFIC GRAVITY OR DILUTION	pH	"TITRATABLE" ACID (CC. N/20 NaOH REQUIRED TO REACH pH 8.3)	pH AT APPROXIMATE POINT OF MINIMUM BUFFER ACTION
A. Diluted juice	Dilution			
17	5 cc. juice + 5 cc. H_2O	5.90	5.1	8.0
17	7 cc. juice + 3 cc. H_2O	5.85	7.3	7.8
17	8 cc. juice + 2 cc. H_2O	5.81	8.4	7.8
17	10 cc. juice + 0 cc. H_2O	5.77	10.4	7.8
B. Natural juice	Sp. gr.			
13	1.0269	5.77	6.2	7.8
16	1.0312	5.74	7.4	7.8
17	1.0461	5.77	10.4	7.8
18	1.0576	5.76	11.9	7.8

Several investigators (**1, 2, 3, 7**) have called attention to the fact that the ampholytes in plant juices may act as buffers, but it is not known whether they are generally present in sufficient quantity to affect the titration appreciably. COHN, GROSS and JOHNSON (**2**) report that between pH 4.5 and 8.5 the form of the titration curve of the juice of potato tubers is largely determined by proteins, principally the globulin tuberin. YOUDEN and DENNY (**13**), however, have found that the proteins have but little effect on the titration of water extracts of this tissue. YOUDEN and DENNY (**13**) found also that a solution of glycocoll containing about the same amount of nitrogen as did the water extract of potato tubers showed comparatively little buffer action. LEUTHARDT (7), on the other hand, concluded that the characteristic form of the titration curve for the juice of *Mesembryanthemum* is due to glutamin, which is present in relatively large quantities.

Removal of the heat-coagulable proteins from wheat juice is easily accomplished by boiling and filtering. According to OSBORNE (9), there probably is very little protein left in an expressed juice after this treat-

ment. The slope of the titration curve for the boiled and filtered juice is slightly steeper than that of the raw, untreated juice (figs. 11, 12, table IV.)[5] The buffering capacity is reduced to practically the same extent in both the acid and alkali titrations. The effect is similar to that shown by

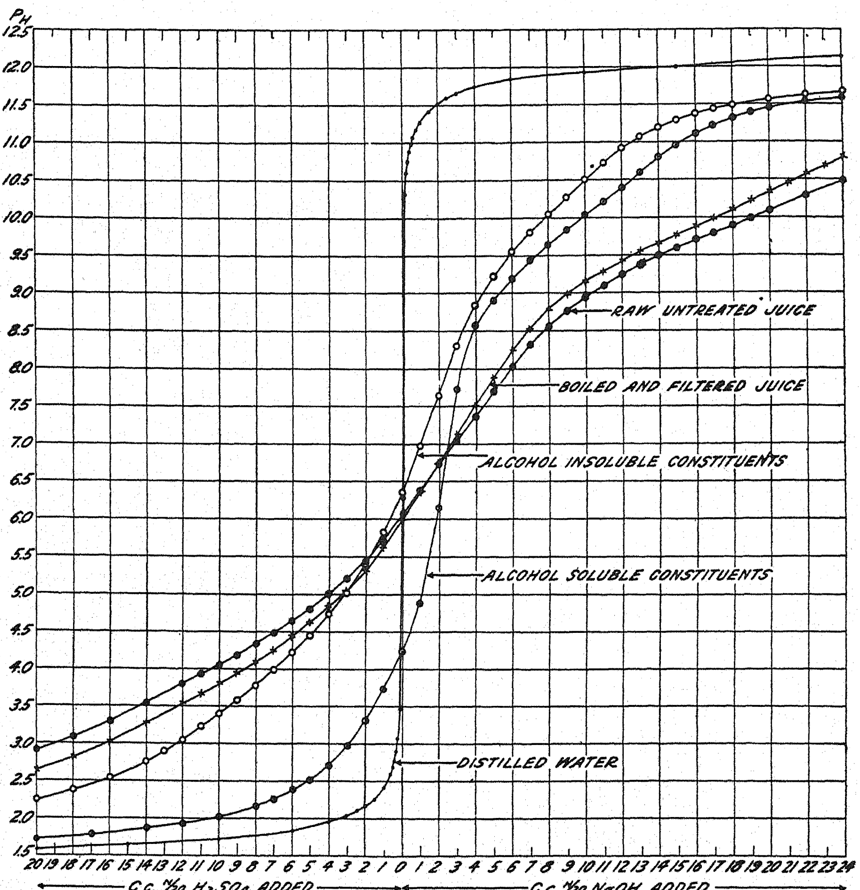


FIG. 11. Titration curves for Jenkin wheat (2 months old) before and after removal of the heat-coagulable and of the alcohol-soluble (hot 95 per cent.) buffers. The curves show that 95 per cent. alcohol dissolves some of the alkali-titratable material but very little of the acid-titratable material.

[5] The data from some of the experiments are presented in the form of graphs (figs. 11–12) and those from others are in tables (tables IV–V). In the case of an experiment such as that of table IV, not all the curves could be drawn in one figure without confusion. Thus the curves for the recombined fractions in the experiments of figs. 11 and 12 had to be omitted because they lay too close to the curve for the whole juice. It seemed desirable, therefore, to give the complete data in the form of tables for one or two typical experiments.

the corresponding curves of YOUDEN and DENNY (13) for an extract of potato tubers, except that these investigators apparently obtained a slightly greater reduction on the alkaline side of the curve than on the acid side. It is evident that the heat-coagulable substances play but a small part in the buffer system of the wheat plant.

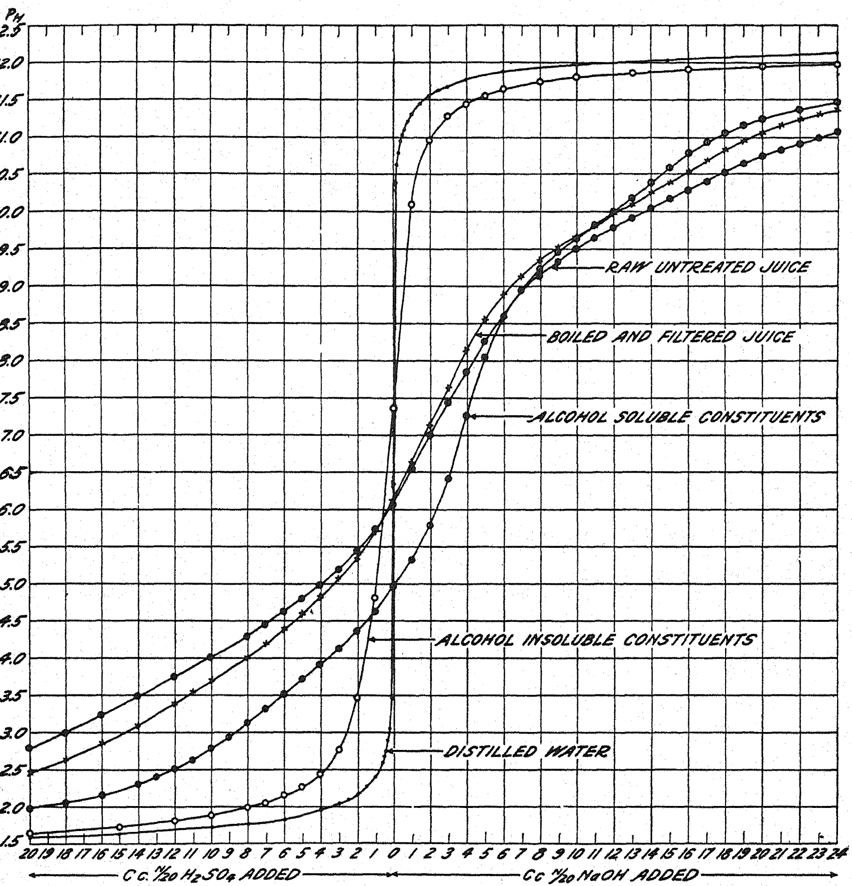


FIG. 12. Titration curves for White Odessa wheat (2 months old) before and after removal of the heat-coagulable and of the alcohol-soluble (cold 75 per cent.) buffers. The curves show that 75 per cent. alcohol dissolves much of both the alkali and acid-titratable material.

Since most of the free organic acids found in plants (8) are fairly soluble in alcohol, they may be largely removed from the juice by evaporating it almost to dryness and extracting with this solvent. However, the salts of these acids are not all soluble in alcohol, so that the alcohol-soluble

fractions could not show the full buffer capacity of the juice. The amino-acids which have been found in plants (8) are, with the exception of proline, but slightly soluble in 95 per cent. alcohol and practically insoluble in absolute alcohol. Polypeptides, also, are but slightly soluble in alcohol. These substances would be left for the most part in the alcohol-insoluble residue after extraction with 95 per cent. or absolute alcohol.

Alcoholic extractions of the evaporated juice of both Jenkin and White Odessa were made. In each case a measured volume of the boiled and filtered juice was evaporated almost to dryness on a steam bath and the residue thoroughly extracted with several portions of neutral redistilled alcohol. The extract was separated from the alcohol-insoluble material by filtration, the alcohol removed from the extract by evaporation on a steam bath, and the residue made up to the original volume with water. The alcohol-insoluble material was then redissolved in the original volume of water. Ten–cc. portions of these fractions were titrated with acid and with alkali. The fractions were then recombined, evaporated, made up to proper volume, and titrated for comparison with the original whole juice.

In one of the experiments reported in table IV, the evaporated juice was extracted successively with alcohol of different concentrations, the different fractions being kept separate, evaporated and made up to volume with water.

Cold absolute alcohol dissolved almost none of the titratable material of the juice, as shown by the fact that the values for this fraction were practically the same as those of distilled water. Cold 95-per cent. alcohol dissolved a little more of the buffer substances, but the curve for this fraction also was similar to that of distilled water (table IV).

Hot 95-per cent. alcohol extracted part of the material which buffers the juice over the alkaline range of the curve, but very little of that which buffers it over the acid range (tables IV, V, fig. 11). The hydrogen-ion concentration of this fraction was always very much higher than that of either the raw juice or the boiled juice, suggesting the presence of acids without their salts or without some of the buffers which are associated with them in the whole juice. The hydrogen-ion concentration of the alcohol-insoluble fraction was always much lower than that of the whole juice—lower even than that of distilled water. This fraction showed almost as great buffer action in the acid range of the curve as did the original boiled and filtered juice, and also considerable buffer capacity in the alkaline range, indicating that not all of the compounds involved in the buffer system are soluble in hot 95-per cent. alcohol.

Seventy-five-per cent. alcohol dissolved practically all of the buffers in the juice as shown by the fact that the shape of the curve for these fractions

TABLE IV

ELECTROMETRIC TITRATIONS OF JUICE FROM WHEAT PLANTS (WHITE ODESSA, 2 MONTHS OLD) BEFORE AND AFTER REMOVAL OF THE HEAT-COAGULABLE BUFFERS, AND OF THE ALCOHOL SOLUBLE AND ALCOHOL INSOLUBLE CONSTITUENTS

Cc. alkali and acid added to 10 cc. juice	Raw, untreated juice	Juice heated to boiling point and filtered	Residue, after evaporation of heated and filtered juice, extracted successively with cold alcohol of different concentrations					Residue extracted with cold 75 per cent. alcohol only			Residue extracted with hot 95 per cent. alcohol only			Distilled water
			100 per cent. alcohol soluble A	95 per cent. alcohol soluble B	75 per cent. alcohol soluble C	Alcohol insoluble D	Recombined fractions A+B+C+D	Alcohol soluble A	Alcohol insoluble B	Recombined fractions A+B	Alcohol soluble A	Alcohol insoluble B	Recombined fractions A+B	
N/20 NaOH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
0	6.09	6.14	4.46	4.19	4.99	7.64	5.04	4.97	7.32	5.30	3.97	6.43	5.64	6.30
1	6.53	6.66	10.80	9.93	5.35	10.87	5.34	5.33	10.08	5.64	4.76	7.11		11.30
2	6.99	7.12	11.32	10.99	5.79	11.32	5.72	5.79	10.94	6.07	6.57	7.80		11.55
3	7.43	7.62	11.54	11.36	6.43	11.51	6.22	6.40	11.26	6.63	8.38	8.54		11.69
4	7.85	8.13	11.65	11.53	7.40	11.63	6.92	7.25	11.43	7.31	9.12	9.08		11.78
5	8.26	8.55	11.73	11.66	8.19	11.72	7.71	8.04	11.52	8.03	9.56	9.50		
6	8.62	8.87			8.75		8.35	8.58	11.63	8.59	9.92	9.81		11.88
7	8.92	9.13	11.84	11.79	9.09	11.83	8.74	8.94		8.94	10.24	10.11		
8	9.14	9.33			9.35		9.00	9.22	11.74	9.20	10.55	10.37		
9	9.34	9.51			9.57		9.23	9.44		9.41	10.84	10.63		
10	9.50	9.66	11.94	11.89	9.76	11.90	9.40	9.63	11.80	9.60	11.06	10.87		11.98
11	9.64	9.80			9.95		9.55	9.80		9.78	11.22	11.06		
12	9.78	9.95			10.17		9.69	9.98		9.92	11.34	11.20		
13	9.90	10.09			10.39		9.87	10.17	11.88	10.09	11.44	11.31		
14	10.03	10.25			10.61		10.06	10.38		10.23	11.52	11.40		
15	10.16	10.39	12.01	11.98	10.81	11.95	10.22	10.59		10.37		11.49		12.04
16	10.27	10.54			10.96		10.43	10.77	11.92	10.52	11.62	11.54		
17	10.40	10.71			11.09		10.62	10.92		10.66				
18	10.52	10.82			11.18		10.78	11.04		10.78	11.69	11.63		
19	10.63	10.94			11.28		10.94	11.14		10.90				
20	10.74	11.05			11.36		11.06	11.23	11.95	11.00	11.74	11.69		
21	10.81	11.14					11.20			11.09				
22	10.89	11.22			11.47		11.28	11.36		11.17				
23	10.98	11.30					11.34			11.24				
24	11.05	11.35	12.08	12.03	11.56	12.01	11.41	11.45	11.97	11.30	11.81	11.78		12.13
N/20 H_2SO_4														
0	6.08	6.15	4.48	4.21	5.02	7.77	5.01	4.97	7.40	5.30	4.01	6.45	5.64	6.25
1	5.73	5.68	2.54	2.61	4.68	3.22	4.69	4.64	4.81		3.38	5.87		2.41
2	5.44	5.33	2.22	2.26	4.40	2.48	4.42	4.36	3.47	4.69	2.89	5.40	4.96	2.16
3	5.19	5.07	2.05	2.09	4.16	2.22	4.19	4.12	2.77		2.55	4.98		2.04
4	4.98	4.82	1.95	1.99	3.94	2.08	4.00	3.91	2.43	4.23	2.33	4.62	4.44	1.95
5	4.80	4.59	1.89	1.91	3.75	1.98	3.80	3.70	2.26	4.05	2.18	4.32		
6	4.62	4.38			3.54		3.63	3.50	2.14	3.87	2.07	4.05	4.03	1.83
7	4.45	4.19	1.79	1.81	3.35	1.85	3.48	3.31	2.05	3.69	1.99	3.80	3.87	
8	4.30	4.01			3.15		3.30	3.12	1.98	3.52	1.93	3.58	3.68	
9		3.85			2.97		3.14	2.93		3.35		3.35	3.51	
10	4.02	3.70	1.69	1.71	2.80	1.74	2.98	2.78	1.88	3.20	1.82	3.14	3.34	1.72
11		3.54			2.65		2.83	2.62		3.05		2.93	3.19	
12	3.75	3.40			2.51		2.69	2.50	1.81	2.90	1.76	2.74	3.04	
13					2.39		2.57	2.40		2.78			2.90	
14	3.49	3.09			2.30		2.47	2.30		2.68		2.47	2.78	
15			1.61	1.62		1.63	2.39		1.73	2.58				1.64
16	3.23	2.86			2.14		2.32	2.16		2.49	1.65	2.27	2.57	
17														
18	3.00	2.63			2.01		2.16	2.03		2.35		2.11	2.41	
19														
20	2.80	2.46	1.54	1.56	1.93	1.57	2.05	1.97	1.64	2.24	1.59	2.02	2.28	1.58

was very similar to that of the whole juice, and further, by the fact that the shape of the curve for the alcohol-insoluble residue remaining after extraction with this solvent closely approached that of distilled water (table IV, fig. 12). The results were practically the same whether the alcohol was hot or cold. It is interesting to note that the fractions containing the substances insoluble in 75-per cent. alcohol were alkaline, the hydrogen-ion concentration being not only lower than that of the original juice, but lower than that of distilled water.

One extraction experiment was carried out with some dried plants of the variety Purplestraw, of approximately the same age and condition as were the White Odessa and Jenkin plants. The plants were dried in an electric oven at 80° C., then ground to a powder in a hand mill. Thirty-five grams of the powder were extracted in 320 cc. distilled water by agitating the mixture for four hours on a shaking machine. The filtrate gave titration values very close to those for a fresh juice. A portion of the filtrate was then evaporated to dryness without previous boiling, and the residue extracted with hot 95-per cent. alcohol. After filtering, the alcohol was removed by evaporation on the steam bath and the residue redissolved in the proper volume of water. The titration values for the solution containing the alcohol-soluble constituents and for that containing the redissolved alcohol-insoluble residue are given in table V, together with the values for the recombined fractions and for the original whole extract. The results are similar to those obtained with fresh juice.

In all the experiments, the hydrogen-ion concentration of the recombined fractions was somewhat higher than that of the original whole juice (table IV) or extract (table V). This increase appeared to be due to changes which occurred during evaporation on the steam bath.[6] However, the buffer action of the extracts was not affected appreciably, as shown by the fact that the curve for the recombined fractions always approached very closely that of the original boiled and filtered juice. That the increase in hydrogen-ion concentration was not due to removal of alcohol-coagulable material was shown by the fact that a similar increase occurred in juice which was evaporated on the steam bath and redissolved in water without treatment with alcohol.

Summary

The electrometric titration curve of juice expressed from wheat plants changes progressively during the seedling stage and during the maturation period. Only minor changes, correlated with environmental factors, occur during the tillering stage and most of the shooting stage.

[6] A sample of juice which was evaporated by boiling at 64° in a partial vacuum and then made up to volume also became more acid. The original pH value of 5.82 was changed to 5.68.

TABLE V

ELECTROMETRIC TITRATIONS OF A WATER EXTRACT OF DRIED WHEAT PLANTS (PURPLESTRAW, 2 MONTHS OLD) AND OF ITS ALCOHOL (HOT 95 PER CENT.) SOLUBLE AND ALCOHOL INSOLUBLE CONSTITUENTS

ALKALI TITRATIONS

CC. N/20 NaOH ADDED TO 10 CC. JUICE	ORIGINAL EXTRACT	ALCOHOL SOLUBLE FRACTION A	ALCOHOL INSOLUBLE B	RECOMBINED FRACTIONS A + B	DISTILLED WATER
	pH	pH	pH	pH	pH
0	5.85	3.89	6.26	5.61	6.30
1	6.33	5.16	6.95		11.30
2	6.83	7.44	7.66	6.53	11.55
3	7.25	8.86	8.60	7.25	11.69
4	7.76	9.48	9.22	7.86	11.78
5	8.35	9.96	9.64	8.33	
6	8.79	10.40	9.90	8.76	11.88
7	9.09	10.79	10.19	9.08	
8	9.34	11.06	10.35	9.33	
9	9.55	11.22	10.54	9.57	
10	9.73	11.36	10.73	9.76	11.98
11	9.92	11.46	10.91	9.92	
12	10.05	11.52	11.05	10.07	
13	10.24		11.17	10.23	
14	10.41	11.63	11.27	10.36	
15	10.58		11.36	10.50	12.04
16	10.73	11.70	11.43	10.65	
17	10.85			10.78	
18	10.98		11.54	10.87	
19	11.10			10.98	
20	11.18	11.79	11.62	11.07	12.09

ACID TITRATIONS

CC. N/20 H_2SO_4 ADDED TO 10 CC. JUICE	ORIGINAL EXTRACT	ALCOHOL SOLUBLE FRACTION A	ALCOHOL INSOLUBLE B	RECOMBINED FRACTIONS A + B	DISTILLED WATER
	pH	pH	pH	pH	pH
0	5.85	3.93	6.24	5.60	6.25
1	5.47	3.16	5.70	5.29	2.41
2	5.18	2.64	5.29	5.04	2.16
3	4.94	2.40	4.98	4.84	2.04
4	4.74	2.22	4.75	4.64	1.95
5	4.56	2.11	4.53	4.48	
6	4.39	2.03	4.35	4.32	1.83
7	4.25	1.96	4.17	4.19	
8	4.10	1.91	4.01	4.05	
9	3.97		3.84	3.91	
10	3.85	1.82	3.68	3.76	1.72
11	3.71		3.52	3.64	
12	3.58	1.75	3.36	3.51	
13	3.44				
14	3.32	1.71	3.06	3.24	
15	3.21				1.64
16			2.79	3.00	
17	2.95	1.66			
18			2.57	2.79	
19					
20	2.65	1.61	2.40	2.62	1.58

The differences in the form of the titration curve for juice of seedlings in successive stages of development indicate differences in composition associated with increasing photosynthetic activity. The end of this period of change in the curve may be interpreted as the end of the seedling stage.

The progressive increase in buffer capacity during the maturation period is due to increasing concentration of the juice. The specific gravity increases regularly during this period, and is closely correlated with the corresponding "titratable acid" values. The initiation of these changes marks the beginning of the maturation period.

Heat-coagulable proteins play a very small part in the buffer system of the wheat plant as shown by the fact that their removal by boiling and filtering reduces the buffer action of the juice only slightly. The buffers remaining in boiled and filtered juice are soluble in either hot or cold 75-per cent. alcohol, partly soluble in hot 95-per cent. alcohol, but practically insoluble in cold 95-per cent. alcohol.

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LIPIDES AND THEIR ESTIMATION IN VEGETABLE TISSUES[1]

CHARLES E. SANDO

When plant tissues are extracted with ether or any of the other fat solvents, the extract contains among other things a large number of substances which yield fatty acids on hydrolysis. The commonest of these and perhaps the most important from a biochemical standpoint are the fats, fatty oils, waxes, sterol esters, phosphatides and cerebrosides. There are also associated with these classes of compounds in various extracts many other substances, some of which are the wax alcohols, sterols, essential oils, hydrocarbons, resins and coloring matters. Some of these accompanying substances are similar to the fats and other fatty acid compounds in possessing a greasy feel but they bear little or no resemblance from a strictly chemical standpoint. Notwithstanding this divergence in chemical makeup, many writers include them in a single large group. It is evident that such a grouping is based almost entirely on the common physical property of being soluble in one or more of the ordinary fat solvents.

Early investigators considered an ether extract of most tissues to consist mainly of neutral fats and other simple glycerides. Later, however, it was definitely established that certain other substances resembling the fats in their solubilities also were removed by ether. To these "fat-like" compounds, the term "lipoids" was applied and is still recognized in this sense by many notwithstanding the fact that the term was first suggested to denote only unsaponifiable substances and has since been extended to include in addition to the "fat-like" substance also the fats and other simple glycerides. Two other terms, "lipins" and "lipides," have also been proposed in connection with the substances under consideration. It will be seen from the following brief discussion of these terms that the nomenclature of the fats and fat-like compounds is still rather confusing.

The term "lipoids" was introduced nearly 70 years ago by KLETZINSKI (**77**) to denote unsaponifiable materials extracted from animal tissues by alcohol and ether. Later, OVERTON (**64**) used the same term to designate those tissue substances which were similar to the fats in being soluble in organic solvents such as ether, chloroform, and alcohol. Since OVERTON's time the term has been employed in several different ways. It has been used in a restricted sense to indicate only the phosphatides and cerebrosides. Reference to fats and lipoids have therefore been made on the assumption that these classes of substances properly belong to separate categories. Many have considered the word "lipoids" a convenient designation for a

[1] A review written at the request of the Committee on Methods of Chemical Analysis for the American Society of Plant Physiologists.

chemically heterogeneous group of substances which are characterized primarily by their physical property of common solubility in one or more of the ordinary fat solvents. LEATHES (**41**) looks upon the term as "a cloak for ignorance and an indefinable limbo into which any one can thrust anything of which he knows little or nothing, including often what is not a compound of any fatty acid at all." Recently, LEVINE (**47**) in proposing a new classification of fatty compounds soluble in ether or alcohol-ether and closely associated substances ignores the broader significance which had been attached to the term "lipoids" and limits its application to the sterols and essential oils.

The designation "lipins" was suggested by LEATHES (**42**) in 1910 for those derivatives of fatty acids which contain nitrogen but no phosphorus or carbohydrate group—an indefinite class of substances previously called "amidolipotides" by THUDICHUM (**87, 88**). Later, ROSENBLOOM and GIES (**73**) employed the term in place of "lipoids" as a group name for the fats and fat-like compounds yielding fatty acids on hydrolysis and also included under lipins a large number of diversified, non-fatty compounds and artificial products such as soaps, alcohols, sterols, cholates, chromolipins, triacetin and lead oleate. McLEAN (**56**) has used the word "lipins" in a limited sense to embrace only the phospatides and cerebrosides which he considers substances of fat-like nature yielding on hydrolysis fatty acids or derivatives of fatty acids and containing in their molecule either nitrogen or nitrogen and phosphorus.

"Lipides" is the third generic name which has been proposed in the general nomenclature of the substances under consideration. It was first suggested and adopted in 1925 by the Committee on the Reform of the Nomenclature of Biological Chemistry at the International Congress of Chemistry meeting at Cambridge (**19**). It was recently used by BLOOR (**6**) in a modified classification in preference to "lipoids" as a group-heading for ether or alcohol soluble compounds yielding fatty acids on hydrolysis and certain derived substances such as fatty acids and sterols. Since this term has been used only in the broad significance to include both the fats (including oils and waxes) and the lipoids, as used in the restricted sense to denote the "fat-like" substances, it appears to be the best one suggested so far.

Various classifications of fats and "fat-like" compounds

A strictly chemical grouping of these substances has proved a difficult matter, especially in view of the diversity of the chemical relationships of the compounds. For purposes of comparison, the more important attempts to classify the fats and "fat-like" compounds are listed here.

THUDICHUM'S CLASSIFICATION, 1884 (87, 88)

Phosphatides or phosphorized principles:

A. **Mononitrogenized monophosphatides** (N: P:: 1: 1)
  lecithins, kephalins, paramyelins, myelins.

B. **Dinitrogenized monophosphatides** (N: P:: 2: 1)
  amidomyelins, amidokephelins, sphingomyelins.

C. **Dinitrogenized diphosphatides** (N: P:: 2: 2)
  assurin.

D. **Nitrogenized phosphatide-sulphatides**
  cerebrosulphatide.

E. **Nonnitrogenized monophosphatides**

Nitrogenized nonphosphorized principles:

A. **Cerebrosides**
  phrenosin, kerasin.

B. **Cerebrinacids**
  cerebrinic acid, spheracerebrin, etc.

C. **Cerebrosulphatides**

D. **Amidolipotides**
  bregenin, krinosin.

ROSENHEIM'S CLASSIFICATION, 1909 (77)

Lipoids:

1. **Cholesterin** group
  cholesterin, phytosterins, lipochromes, etc.
2. **Cerebro-galactosides**
  phrenosin, kerasin.
3. **Phosphatides**
  Monamino-monophosphatides (N: P:: 1: 1)
    lecithins, kephalins, vesalthin.
  Diamino-monophosphatides (N: P:: 2: 1)
    sphingomyelin.
  Triamino-monophosphatides (N: P:: 3: 1)
    neottin.
  Triamino-diphosphatides (N: P:: 3: 2)
  Monoamino-diphosphatides (N: P:: 1: 2)
    cuorin.

LEATHES' CLASSIFICATION, 1910 (42)

Substances entering into the composition of fats:

A. **The fatty acids**

B. **Glycerol and the glycerides**
  fats and oils.

C. **Other alcohols and their fatty acid esters**
  cetyl alcohol, myricyl alcohol, cholesterol, phytosterol, waxes, esters of cholesterol, etc.

D. **Phospholipins**
  compounds of fatty acids containing N and P., *e.g.*, lecithin.
  Galactolipines
  compounds of fatty acids containing N and galactose, *e.g.*, cerebrone.

Lipines
compounds of fatty acids containing N but no P nor carbohydrate group, *e.g.*, **sphingosine.**

BANG'S CLASSIFICATION, 1911 (2)

Lipoids:
1. **Fats**
2. **Cholesterols**
3. **Phosphatides**
    A. **Unsaturated phosphatides**
        Monaminomonophosphatides
            lecithin, kephalin, paramyelin, vesalthin.
        Monaminodiphosphatides
            cuorin, etc.
        Triaminodiphosphatides
            sahidin, etc.
    B. **Saturated phosphatides**
        Diaminomonophosphatides
            sphingomyelin, aminomyelin, apomyelin, etc.
        Triaminomonophosphatides
            neottin, carnaubon.
        Protagon
4. **Cerebrosides**
        phrenosin, kerasin, cerebron, etc.

CRAMER'S CLASSIFICATION, 1911 (12)

Lipoids (with the exception of the cholesterols):
1. **Phosphatides**
    N-containing fatty acid esters of glycero-phosphoric acid. Some may have the glycerol substituted by an unknown alcohol. Lecithin, kephalin, sphingomyelin, etc.
2. **Galacto-phosphatides**
    N-containing fatty acid esters of phosphoric acid, with galactose and alcohol groups. Carnaubon.
3. **Cerebrosides**
    N-containing fatty acid esters with galactose but no P. Cerebron, cerebrin, homocerebrin, etc.
4. **Phospho-cerebrosides**
    cerebrosides with P-containing groups. Protagon.

ROSENBLOOM AND GIES'S CLASSIFICATION, 1911 (73)

Lipins, organic substances insoluble in concentrated saline solutions, soluble in hot alcohol or in warm ether or in both.
1. **Natural aliphatic lipins**
    A. **Simple lipins**
        a. **Fatty acids**
        b. **Salts and esters of 1-A-a**
            soaps, waxes, fats and fatty oils.
        c. **Alcohol** (mono- and di-hydroxy)
            cetyl alcohol, myricyl alcohol.

B. **Conjugate lipins**

a. **Proteolipins**

lecitho-protein like substances (may be mixtures).

b. **Glycolipins**

THUDICHUM'S cerebrosides.

c. **Phospholipins**

THUDICHUM'S phosphatides.

d. **Glyco-phospholipins**

phospholipins containing carbohydrate group.

2. **Natural carbocyclic lipins**

A. **Sterols**

natural terpeno-alcoholic derivatives, nonsaponifiable, and form esters, cholesterol, sitosterol, etc.

B. **Esterols**

natural terpeno-aliphatic waxes, *e.g.*, cholesterylpalmitate

C. **Cholates**

a. Cholic acids

b. Bile acids

3. **Natural lipins of undetermined constitution**

A. **Chromolipins**

B. **Miscellaneous lipins**

4. **Artificial lipins**

tri-acetin, lead oleate, etc.

MATHEW'S CLASSIFICATION, 1914 (54)

Lipins—constituents of protoplasm with greasy feel; soluble in alcohol-ether.

1. **Fats**

Fatty acids

2. **Fatty oils**

drying oils, semi-drying oils, non-drying oils.

3. **Essential oils**

volatile, generally odoriferous substances of oily and of varied chemical nature, being aldehydes, acids, terpenes, alcohols, etc.

4. **Waxes**

esters of sterols and fatty acids.

5. **Sterols**

alcohols, generally of the terpene group, solid at ordinary temperature; oxidation products are terpenic acids.

6. **Phospholipins** (phosphatides of THUDICHUM)

Mono-amino-monophospholipins, lecithin, cephalin.

Di-amino-monophospholipins

Mono-amino-diphospholipins

7. **Glycolipins** (cerebrosides of THUDICHUM), cerebron, phrenosin, etc.
8. **Sulpholipins**—not well characterized.
9. **Aminolipins**—not well characterized.

MACLEAN'S CLASSIFICATION, 1918 (56)

Substances in an ether or alcohol extract of a tissue:

A. **Neutral fat and fatty acids**

B. **Substances of varying chemical nature having no relation to fat** such as cholesterol and certain pigments.

Lipins: "fat-like" bodies, sometimes referred to as "lipoids"

C. **Phosphatides**

1. **Monoaminomonophosphatides** (N: P:: 1: 1), lecithin, kephalin.
2. **Monoaminodiphosphatides** (N:P::1:2), cuorin.
3. **Diaminomonophosphatides** (N:P:: 2:1), sphingomyelin.

D. **Cerebrosides**

phrenosin, kerasin.

LEVINE'S CLASSIFICATION, 1925 (47)

True lipins, compounds soluble in ether or alcohol-ether yielding on hydrolysis fatty acids and alcohol.

1. **Simple lipins,** compounds which yield on hydrolysis, fatty acid and glycerol or fatty acid and a monohydric alcohol of high molecular weight.
   a. **Fats**
   b. **Fatty oils**
   c. **Waxes**
2. **Conjugated lipins,** compounds which yield on hydrolysis, not only fatty acid and alcohol but some other complex such as sulphuric acid, phosphoric acid, monosaccharide, amino-acid, or some organic base like choline.
   a. **Phospholipins, lecithin,** cephalin, sphingomyelin.
   b. **Glycolipins,** cerebrin, phrenosin, kerasin.
   c. **Glycophospholipins,** jecorin.
   d. **Sulpholipins**
   e. **Sulpho-phospholipins**
   f. **Aminolipins,** bregenin.
   g. **Proteolipins**
   h. **Chromolipins**
3. **Derived lipins,** compounds other than phosphoric acid, sulphuric acid, amino acid or monosaccharide, obtained as a result of the decomposition of lipins.
   a. **Fatty acids**
   b. **Alcohols,** glycerol, myricyl alcohol, etc.
   c. **Organic bases,** choline, neurine, etc.

Lipoids, compounds not esters of fatty acid and alcohol, but which are closely associated with lipins and resemble them in their solubility in ether or alcohol-ether.

1. **Sterols,** cholesterol, phytosterol, etc.
2. **Essential oils**

BLOOR'S CLASSIFICATION, 1925 (6)

Lipides, substances having the following characteristics: (a) insolubility in water and solubility in fat solvents, such as ether, chloroform, benzol, (b) relationship to the fatty acids as esters, either actual or potential, (c) utilization by living organisms.

Simple lipides. Esters of the fatty acids with various alcohols.

Fats

Waxes

Compound lipides. Esters of fatty acids containing groups in addition to an alcohol and fatty acid.

- **Phospholipides,** containing phosphoric acid and nitrogen; lecithin, cephalin, sphingomyelin.
- **Glycolipides,** containing carbohydrate and nitrogen but no phosphoric acid; cerebrosides.
- **Aminolipides, sulpholipides,** etc., not at present sufficiently well characterized for classification.

Derived lipides. Substances derived from the above groups by hydrolysis.

- **Fatty acids**
- **Sterols,** mostly high molecular weight alcohols, found in nature combined with fatty acids and which are soluble in the fat solvents; cholesterol, myricyl alcohol, etc.

It is not within the scope of this paper to discuss the relative merits of the various classifications, but it must be noted in passing that a great deal of confusion and difference of opinion exists in the literature as to what properly constitutes a single group of closely related fatty compounds and as to what terms should be used in their designation.

In the present paper, the use of the term "lipides" is preferred because of the fact that the several meanings which have been attached to the other two terms tend to create a state of disorder in an already difficult subject and therefore constitute an objection which does not apply in the case of "lipides." On the basis of chemical relationships, the BLOOR scheme appears more nearly to meet the requirements of a good classification than the others. The writer does not agree with BLOOR, however, in always considering the sterols as being derived from ester-like compounds.

According to BLOOR's definition, lipides are water insoluble, ether or alcohol soluble substances which are either ester-like combinations with fatty acids or are capable of forming such combinations. These substances are separated into three main groups. The fats and waxes are considered as simple lipides, being esters of the fatty acids with various alcohols. The phospholipides, glycolipides and certain insufficiently characterized substances constitute the compound lipides or esters of fatty acids containing groups in addition to an alcohol and fatty acid. The free fatty acids and sterols represent the derived lipides, which are defined as substances obtained from the other groups by hydrolysis. For a thorough discussion of the various types of fatty compounds which are included in the foregoing classification and for their physical and chemical properties, one may refer to BLOOR's original paper (**6**).

A great deal of confusion would be avoided if plant physiologists and biochemists in general would adopt the BLOOR scheme and designate as lipides those tissue constituents which fall under this classification. When obtained from tissues in the form of an impure extract by means of a fat solvent they could be reported as "crude lipides."

The estimation of lipides

Nearly all methods for determining lipides are based on the fact that these substances, or the fatty acids derived therefrom by saponification, are largely separated from the other tissue constituents by means of certain solvents which exert at least a partial preferential solubility for the fatty compounds. Petroleum ether, especially, and ether, to a less degree, are better suited for this purpose and therefore are used more extensively than other solvents for lipide estimation. While all lipides are more or less soluble in a number of so-called fat solvents, not all of these extract the lipides with equal facility (**37**) or extract the same non-lipide substances. With dry oat kernels (**5**), for example, if the total 15-hour ether extract is considered equivalent to 100, the quantities of material extracted by other fat solvents are as follows: petroleum ether 97.07, carbon tetrachloride 104.24, chloroform 109.78, acetone 112.71, benzene 113.15 and absolute alcohol 127.93. In this particular case petroleum ether, of all the solvents, removes the smallest quantity of extract, while absolute alcohol removes the largest. It should be recognized that in most instances the extract obtained from plants by the use of petroleum ether contains the least quantity of non-lipide substances. Ether, carbon tetrachloride, chloroform, and the other solvents are all known to extract increasing quantities of impurities such as resins, alkaloids, coloring matters and other organic substances. Regardless of the fact that petroleum ether is probably the more suitable solvent to use for the extraction of lipides in many cases, many plant physiologists use ether instead when extracting dried plant tissues. In fact, this has become the most generally used fat solvent in the laboratory. However, in the crude lipide extract obtained from many plant tissues by the use of ether the non-lipide substances may amount to more than the total weight of true lipides. This fact introduces a considerable error in case the extract is reported as "crude fat" or "lipoids" as is very often done by investigators working in agronomic and horticultural fields.

Next to the careful selection of solvent it is very important that attention be paid to the purity of that solvent. It is well known, for example, that impure ether, containing water and alcohol, will yield a greater quantity of extract than the pure anhydrous solvent yields. Such extracts are contaminated with more non-lipide substances than extracts obtained by the use of a pure solvent.

The foregoing statements are sufficient to emphasize the importance of the solvent in the extraction and estimation of lipides from vegetable tissues. On this account, it seemed desirable to include in this paper general data dealing with the common fat solvents[2], including a table of physical

[2] Compare also the following: (**53, 8, 97, 52**).

constants. It must be remembered, however, that due to accompanying substances which exert an influence on their behavior, the solubilities of the pure lipides in pure solvents do not necessarily hold when lipides are extracted from tissues.

LIPIDE SOLVENTS

PETROLEUM ETHER (C_nH_{2n+2}).—Petroleum ether (b. p. 40–60°) readily dissolves most fats, oils, waxes, phospholipides, fatty acids and sterols; some of these even in the cold. Hydroxy acids and glycerides of these acids, for example, castor oil, are insoluble or nearly so in this solvent. Glycerides of the solid fatty acids are more difficultly soluble than those of the liquid acids. Pure tristearin, therefore, is only slightly soluble, but it becomes more so in the presence of soluble glycerides. Of the fatty acids, those of higher melting point are the least soluble. Hydrocarbons, essential oils, certain coloring matters to a slight extent, such as chlorophyll "a" and carotin, and a few other organic substances are soluble in petroleum ether. Despite the fact that this solvent dissolves certain non-lipide substances, it may be said to remove from plant materails the least quantity of these impurities in comparison with other solvents. This is particularly true with respect to coloring matters and compounds of a resinous nature.

ETHYL ETHER (C_2H_5–O–C_2H_5).—Practically all lipides are fairly readily soluble in ether with the exceptions of sphingomyelin and the cerebrosides. Fats are more easily soluble the higher the content of glycerides of unsaturated fatty acids and those of low molecular weight. Pure tristearin is difficultly soluble but is more so in the presence of other glycerides. Lecithin is generally considered soluble but its complete removal from plant tissues with ether, especially from leguminous seeds is difficult. The extraction of this and similar substances which are but slowly removed even on prolonged extraction is greatly facilitated by alcoholic pre-treatment (7). It is generally known that ether dissolves more non-lipide materials from plant tissues than petroleum ether. Among the soluble substances may be mentioned hydrocarbons, essential oils, coloring matter (including chlorophylls, carotinoids, free flavones and flavonols), many alkaloids, organic acids, resins and related compounds. CHIBNALL and CHANNON (**11**) state it has been their experience that the presence of a small amount of water in ether will allow a small but definite amount of inorganic matter and amino-compounds to go into solution. The same statement holds true with respect to carbohydrates and possibly tannins.

CARBON TETRACHLORIDE (CCl_4).—Carbon tetrachloride dissolves most lipides with the same ease as ether, but it probably removes more impurities (**70**). The observation of SCHINDELMEISER (**80**) that many alkaloids are soluble in cold carbon tetrachloride indicates that these substances would

TABLE I

TABLE OF CONSTANTS FOR PURE FAT SOLVENTS[3]

SOLVENT	BOILING POINT	SPECIFIC GRAVITY	VAPOR PRESSURE (mm. Hg.)		HEAT OF VAPORIZATION		SOLUBILITY	
			AT 20°	AT 30°	cal./gm.	K cal./mol.	SOLVENT IN WATER	WATER IN SOLVENT
Petroleum ether							At 22° = 0.227 per cent.	At 22° = 0.50 per cent.
n-pentane	36.3°	.6454$^{0}/_{4}$	420.2	610.9	85.0		(Petroleum ether of sp. gr. = .6646)	
n-hexane	68.6°	.6794$^{0}/_{4}$	120.0	185.4	79.2	7.80		
Ether	34.6°	.7191$^{15}/_{4}$	538.0	914.0	84.5	6.26	At 20° = 1.22 per cent. At 30° = 1.25 per cent.	At 20° = 6.48 per cent. At 30° = 5.04 per cent.
Carbon tetrachloride	76.7°	1.6319$^{0}/_{4}$	91.3	141.1	46.4	7.14	At 20° = 0.08 per cent. At 30° = 0.085 per cent.	
Chloroform	61.0°	1.4989$^{15}/_{4}$	158.4	240.0	61.4	7.35	At 20° = 0.617 per cent.	At 20° = 0.10 per cent.
Trichlorethylene	88.0°	1.47$^{15}/_{15}$	56.0	92.0	58.0		At 22°, 100 cc. of water dissolves 0.08 gm. or 0.5 cc.	
Carbon disulphide	46.3°	1.2922$^{0}/_{4}$	298.0	717 (40°)	86.7	6.60	At 22° = 0.218 per cent.	At 22° = 8.76 per cent.
Benzene	79.8°	.8799$^{20}/_{4}$	75.0	118.0	94.9	7.41	At 25° = 0.113 per cent.	At 25° = 0.023 per cent.
Acetone	56.6°	.8125$^{0}/_{4}$	184.8	282.7	125.0	7.26		
Alcohol	78.3°	.7093$^{15}/_{4}$	44.0	78.06	216.5			

[3] Compiled from the following: (40, 97, 8).

be readily removed from plant tissues on continuous extraction with the hot solvent. For a more detailed account of the solvent properties, refer to BASKERVILLE and RIEDERER (3). BRYANT (10) claims carbon tetrachloride extracts the fats and similar substances in a much shorter time than is required for some of the other solvents. His results seem to indicate that this solvent removes in 2 hours the same quantity of fat (lipides) obtained in 16 hours by the use of ether and in 4 hours by carbon disulphide. WILEY (94) says "carbon tetrachloride would be a most desirable solvent, owing to the fact that it is not inflammable and that it dissolves nearly all the fats and oils with ease, if it were not that the last traces of it are removed with the greatest difficulty and also that in the presence of even minute traces of moisture it is partly decomposed into hydrochloric acid, which of course may act upon the fat and make it useless for further determinations."

CHLOROFORM ($CHCl_3$).—Chloroform is a good solvent for most lipides. However, it dissolves the same and probably more non-lipide substances than ether. The fact that it dissolves more substances than ether does is readily shown by extracting plant materials exhaustively with ether and then following with chloroform, in which case the chloroform will be found to remove a further appreciable quantity of soluble substances. SCHLESINGER (81) and ROSENFELD (76) use chloroform as solvent in lipide determination methods. In the ROSENFELD method, the material is first heated on the water-bath with alcohol, then extracted in a continuous extractor with chloroform. Finally the dry residue, obtained after the evaporation of the solvent, is extracted with absolute ether. KUMAGAWA and SUTO (38) have pointed out that the energetic solvent involved in the more efficient removal of lipides in this method is mainly the alcohol and not the chloroform.

CARBON DISULPHIDE (CS_2).—This solvent is a liquid of high solvent power for a wide range of substances. It readily dissolves oils, fats, waxes, resins, and many other organic substances. It has not come into general use for quantitative lipide estimation, probably because of its poisonous properties, its unpleasant odor and its liability to decomposition when used for the extraction of certain lipides.

BENZENE (C_6H_6).—Benzene is a good solvent for fats, oils, waxes, sterols and phospholipides. Cerebrosides are soluble in the hot solvent. Fatty acids in general are easily soluble and to a higher degree than in petroleum ether. Here also the solid fatty acids are less soluble than the liquid unsaturated ones. For example, oleic acid is soluble in all proportions, but stearic acid only to the extent of 0.22 parts in 1 of benzene at 23° C. Soaps are taken up in not inconsiderable quantities if free fatty acids or neutral oils are present. Benzene dissolves hydrocarbons, certain coloring matters, resins and many other organic compounds. Last traces of benzene are very difficult to remove from a lipide residue.

TRICHLORETHYLENE (C_2HCl_3).—This solvent dissolves most lipides very readily, but it also dissolves many other organic compounds, among which may be mentioned (**21**) benzoic, salicylic, and cinnamic acids, acetaldehyde, benzaldehyde, vanillin, camphor, alizarin, asparagine, glycine, caffeine and theobromine. Many of these non-lipide substances are doubtless left behind when the lipides in a sample are being determined by simple shaking with cold trichlorethylene and evaporation of an aliquot part of the fat solution.

ACETONE (CH_3–CO–CH_3).—Acetone has a wide range of solvent power for many classes of organic compounds. The fats, oils, and waxes are only slightly soluble in the cold, but readily soluble in the hot solvent. The cerebrosides and fatty acids are soluble, but lecithins and cephalins are practically insoluble. Sphingomyelin is insoluble or nearly so in cold but somewhat soluble in hot acetone. Hydrocarbons, sterols, many resins, coloring matters, certain glucosides, tannins and other compounds are all more or less soluble in this solvent. Its solvent action is too general to permit its use for quantitative work. It has been recommended in place of alcohol to extract the lipides where the residue obtained is subsequently to be extracted with ether or petroleum ether to separate impurities.

ALCOHOL (C_2H_5OH).—Alcohol is a most excellent and energetic solvent for many classes of organic compounds. In this respect it may be said to possess a wider range of solvent power for plant constituents than any other solvent with perhaps the single exception of water. It is known to extract many compounds belonging to the following classes: lipides, carbohydrates, alkaloids, glucosides, tannins, saponins, resins, organic acids, bases, proteins (a few are soluble), coloring matters, etc. With respect to fats, oils, and waxes it may be stated that they are only slightly soluble in cold, but are more readily dissolved in the hot solvent. The solubility of fats and oils varies with the nature of the combined fatty acids. Glycerides of lower, unsaturated fatty acids being more soluble than those of the higher, saturated ones. LEWKOWITSCH (**48**) points out that the solubility of most fats and oils in absolute alcohol at 15° does not exceed 2 per cent. while in 95 per cent. alcohol the solubility is still less. Castor oil is a notable exception, being readily soluble in all proportions at ordinary temperature. Palmitin, stearin, and olein are almost insoluble in 91 per cent. alcohol. At 35° myristin is little soluble, while laurin is easily soluble. At higher temperatures the solubilities increase. The presence of free fatty acids also influences solubility, the presence of 50 per cent. making solution complete. Free fatty acids are soluble in alcohol, although from palmitic upwards all are sparingly soluble. For example, oleic acid and most unsaturated acids are readily soluble, but only 9.3 parts of palmitic and 2.5 parts of stearic acid are soluble in 100 parts of alcohol. Cerotic acid is almost insoluble at 20°,

but soluble in boiling alcohol. The majority of oils and fats are soluble in absolute alcohol. In the use of alcohol at its boiling point for the removal of lipides, there is some liklihood of loss due to decomposition of the more sensitive lipides. The use of alcohol under reduced pressures as a means of extracting lipides from vegetable tissues without decomposition is well worthy of consideration. The possibility of the solvent reacting with the dissolved fatty acids at the ordinary boiling point of alcohol must not be overlooked in the use of this solvent to extract plant materials. HOLLAND (**30**) claims boiling with alcohol causes a drop in the neutralization number of fatty acids, particularly stearic and myristic acid. It was observed by EMERSON and DUMAS (**17**) that 0.3 per cent. of palmitic acid was esterified in the short time necessary for the solution of this acid in alcohol on the water-bath and the evaporation of the alcohol. Of the phospholipides, cephalin is characterized by its insolubility in alcohol. Sphingomyelin is only slightly soluble in the cold, but soluble in hot alcohol. Lecithins are soluble. The glycolipides (cerebrosides) and sterols are readily soluble in hot alcohol.

CRUDE LIPIDE DETERMINATION METHODS

The principles involved in the extraction of lipides are practically the same whether one is dealing with animal or plant tissues. We may therefore conveniently separate the more important methods for the estimation of lipides in both classes of material into two general groups.

FIRST GROUP.—In the first group may be placed those methods which are based on the removal of lipides from the tissues in a condition as little altered as possible. The methods in this group may be subdivided into (a) those based on the direct extraction of the material with a fat solvent or a combination of solvents, and (b) those in which the material is subjected to a mild pretreatment such as pepsin digestion, or treatment with dilute acid or alkali, followed by extraction with a fat solvent.

The more important methods under "a" are those of shaking with ether, petroleum ether, or trichlorethylene according to LOGES (**51**), SCHÜTTE (**83**), MONHAUPT (**57, 58, 59**), GRIMME (**22**), NEUMANN (**61, 62**, *cf.* also PHILLIPS, **68**) and GROSSFELD (**23, 24, 25, 26**); the ether extraction methods of SOXHLET (**85**, *cf.* also BÖMER, **9**) (with modifications adopted as the "Official method" by the Association of Official Agricultural Chemists (**96**)), LEHMANN (**44**) and VÖLTZ (**91**); the petroleum ether extraction methods of GLIKIN (**20**), LEPPER and WATERMAN (**46**); the carbon tetrachloride extraction method of BRYANT (**10**); the alcohol-ether, alcohol-petroleum-ether, or alcohol-chloroform methods of BOGDANOW (**7**), FRANK (**18**), VOIT (**90**), NOEL-PATON (**63**), HERTWIG (**29**), ROSENFELD (**74, 75, 76**); and the modified

KOCH method (applicable to fresh tissues (**34, 35, 36**)) (*cf.* also APPLEMAN (**1**), CULPEPPER, FOSTER and CALDWELL (**13**), JONES (**32**), WALSTER (**92**), and SCHERTZ (**79**)).

Under "b" are placed the acid-pretreatment methods of PFLÜGER (**67**), PALMQUIST (**66**), POLENSKE (**69**), WEIBULL (**93**); the ammoniacal-alcohol method of RASK and PHELPS (**71**); and the pepsin-digestion method of DORMEYER (**15, 16**) (*cf.* also SCHLESINGER (**81**), BEGER (**4**), MÜLLER (**60**), and DIESSELHORST (**14**)).

SECOND GROUP.—In the second group may be placed those methods which are based on the extraction of the fatty acids produced on saponification of the lipides originally present in the tissues. This group includes the methods of LIEBERMAN and SZÉKELY (**49, 50**) (*cf.* also LEATHES (**43**), ROSENTHAL and TROWBRIDGE (**78**), KUMAWAGA and SUTO (**37**), *cf.* also KUMAWAGA (**39**), INABA (**31**), SCHIMIDZU (**84**), LEMLAND (**45**), and TERROINE, LEPAGE, VÉCHOT and WOLFF (**86**), and of RATHER (**72**)).

Not all of the methods included in the two foregoing groups have been employed for plant tissues. It is therefore not known whether they may be used without further modifications to meet the special needs of plant materials. Of those methods which are applicable to vegetable tissues only the three following will be discussed in detail.

1. OFFICIAL METHOD OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (96)

ABSTRACT OF METHOD.—Two or more grams of properly dried and ground material are extracted for 16 hours with anhydrous ether. The extract, after evaporation of the ether, is dried for 30 minutes at the temperature of boiling water, cooled in a desiccator and weighed. The drying is continued for 30-minute periods until the weight of the residue remains constant.

DETAILS OF OPERATION.—For the extraction of lipides from solid plant materials, according to the "Official method," the Soxhlet apparatus or one of its many modifications is generally used, although any of the numerous types of continuous extractors[4] which are now available may serve equally well. The quantity of dried material to be extracted will vary from 2 to 10 grams or even more, depending upon the percentage of fatty substances contained therein. The sample, carefully selected for uniformity, should be dried, preferably at 60° in a partial vacuum, and finely ground.[5] The accurately weighed substance is placed either in a Schleicher and

[4] For a discussion of automatic devices for the extraction of powdered material see WILEY (**95**) and PALKIN and WALKINS (**65**).

[5] See recommendations of Committee on Methods of Chemical Analysis for American Society of Plant Physiologists (**89**).

Schüll fat-free paper thimble, in a Schott and Company "glass filter" (carried by the Empire Laboratory Supply Company) or in a porcelain cup (**27**) and covered with a fat-free paper or cotton. The thimble and sample are placed in the extractor[6] and elevated, if necessary, by means of a glass tube of sufficient heighth so that the surface of the material is about 1 cm. under the top of the siphon tube. This adjustment insures a more complete extraction of the sample.

The flask, containing several glass beads to prevent bumping of the solvent during extraction, is thoroughly dried by heating, cooled over sulphuric acid in a desiccator, and weighed with the aid of a counterpoise of nearly the same size and shape. A portion of the solvent, about 50 to 150 cc., is poured into the flask, which is then connected with the extractor holding the sample. A further quantity of solvent is carefully poured over the material in the extractor until the height of the liquid nearly corresponds to the level of the top of the siphon tube. A tube containing anhydrous calcium chloride to prevent absorption of water by the solvent is attached to the top of the condenser.

The solvent is boiled during the period of extraction, either on an electric hot-plate or on a steam bath. The vapors of the solvent liquify in the condenser, the condensate flows into the extractor, penetrates the material and dissolves the lipides. When the liquid reaches the top of the siphon tube, it should automatically return to the flask. With some materials it is desirable to discontinue the extraction after the greater portion of lipides has been extracted and to reduce the sample to a finer state for a second and final period of extraction. The material, after drying to remove traces of the solvent, is ground in a mortar with 1/2 to 2/3 of its weight of pure quartz sand and the finely ground mixture quantitatively replaced in the thimble. Final traces in the mortar are obtained by removing with fat-free cotton, which must be added to the main sample in the thimble. The extraction is then continued as before. In some plant tissues the removal of lipides is nearly complete in 4 to 6 hours, in others 16 hours or longer are required. No definite time limit can be prescribed in all cases. This must be experimentally determined. In general, it may be stated that the size of particles and degree of porosity of sample are important factors influencing the rapidity and completeness of the extraction with any particular solvent.

Removal of the solvent from the extraction flask upon completion of the extraction is easily performed by using the apparatus itself for the distillation. After removal of the sample and glass tube, the boiling is continued until most of the solvent is distilled into the extractor. If necessary the solvent is siphoned off into a second container and the process continued

[6] Directions indicated apply to the Soxhlet apparatus.

until nearly all of the liquid in the flask has been evaporated. The final portions of solvent must be evaporated on a steam bath, care being exercised to prevent any spattering of the contents of the flask. Last traces of the solvent may be eliminated with a stream of pure dry carbon dioxide.

The residue in the flask is dried for 30 minutes at the temperature of boiling water, cooled over sulphuric acid in a desiccator and weighed. The drying is continued for 30-minute intervals, the flask being cooled and weighed each time, until the weight remains constant. If the material is very hygroscopic it may be necessary to dry at room temperature over calcium chloride in a vacuum desiccator until constant weight has been attained. If exactly 10 grams of dried substance are used as the original sample, the weight of the residue multiplied by 10 gives directly the percentage of fatty residue. This is sometimes reported as "fat" or "crude fat." It would be more nearly correct, however, to express the results as "crude lipides" or "ether extract."

General remarks.—When considering the ether extraction of dried samples according to the "Official method," it should be borne in mind that a portion of the lipides present in the fresh tissues inevitably suffers partial decomposition during desiccation. If the drying is carried out at the temperature of boiling water, and with exposure of the material to air, changes occur due to the partial oxidation of compounds containing unsaturated fatty acids. Such changes are minimized but not entirely eliminated if the drying is accomplished at reduced pressures or in an atmosphere of neutral gas.

Another error in the ether extraction method is due to the varying quantities of impurities which are removed from the tissues along with the true lipides and reported as such. The determination of lipides in cacao products furnishes an illustration. Here the use of ether results in the extraction of significant quantities of the alkaloid, theobromine. Other impurities such as hydrocarbons, coloring matters, and resin compounds are sources of error. Many of these may be determined and corrected for, where the quantity of residue is sufficient to justify an examination for unsaponifiable matter. In plant physiological work one is often limited with respect to quantity of available material, consequently the residue obtained from an ether extraction is usually too small to attempt further examination. In such cases it is desirable to have the residue represent as pure an extract of lipides as possible.

It is generally recognized that a small loss of lipides occurs through incomplete extraction. A portion of the lipides either becomes firmly entangled with carbohydrate and protein matter during the drying of the original sample, or the solvent is unable to penetrate the material properly

within a reasonable length of time. That the removal of ether soluble constituents is nearly always incomplete was shown by PFLÜGER (**67**) and by DORMEYER (**16**) in the case of dried animal materials. The difficulties experienced in removing lipides from plant tissues are not as serious as in the case of animal materials, since it is well known that lipides are held more tenaciously in animal tissues. SCHULZE and STEIGER (**82**) and MAXWELL (**55**) reported that phospholipides (phosphatides) can be completely removed from seed material only if the ether extraction is followed by successive extractions with alcohol at 60°. That an ether extraction of plant materials may be incomplete has also been pointed out by RATHER (**72**), HERTWIG (**28**) and by RASK and PHELPS (**71**).

2. MODIFIED KOCH METHOD[7]

ABSTRACT OF METHOD, FIRST PROCEDURE.—A sample of fresh material, weighing from 50 to 150 grams, is introduced into a sufficient quantity of boiling 95 per cent. alcohol to insure a final concentration of at least 80 per cent., after allowance for the original water content of the tissue. The mixture is boiled 15 minutes. The cold alcoholic liquid is filtered through extraction cups, and the residue exhaustively and successively extracted with alcohol, ether, and again with alcohol. The first alcoholic preserving filtrate and the combined alcohol and ether extracts are added together and evaporated to dryness. The residue is then thoroughly extracted with anhydrous ether. The extract, after the evaporation of ether, is dried to constant weight. The dried residue represents "crude lipides."

DETAILS OF OPERATION.—The fresh plant material to be analyzed is collected, rapidly cut into small pieces or otherwise reduced to finely divided state and thoroughly mixed to insure uniformity. Immediately after the material has been mixed, 50 to 150 gram duplicate samples are quickly and accurately weighed out and introduced into sufficient redistilled 95 per cent. boiling alcohol to bring the final concentration to at least 80 per cent., after allowance for the water originally present in the tissue. Boiling is continued for 15 minutes to arrest enzyme activity. Preserved in this manner, the material may be stored several months before the analytical work is begun, although it might be advisable to reheat the samples at least once or twice during the storage period.

[7] The original KOCH method was first employed in the analysis of animal tissues by WALDEMAR KOCH (34, 35, 36). Later F. C. KOCH, University of Chicago, introduced changes which were embodied in his unpublished student outline for the analysis of tissues. Students of plant physiology have made further modifications (1, 13, 32, 92, 79). The modified KOCH method provides for the separation of the constituents of tissues into a number of fractions which are separately examined. In the present paper discussion is confined to the so-called "lipoid" fraction.

For the extraction of the alcohol-preserved material, the insoluble residue is quantitatively transferred to Schleicher and Schüll fat-free paper extraction thimbles or to porcelain cups which are perforated on the bottom and fitted with filter-paper. If paper thimbles are used they may be placed upright in funnels in such a way that the liquid drips through the cups into flasks. The procelain cups are suspended by clamps over the flasks or beakers to accomplish the same purpose. In transferring the contents of the containers into the cups, the greater part of the material is poured into a beaker and the supernatant liquid from this used to transfer adhering matter from the container to the cup. The whole sample is then carefully removed to the cups, the last trace of substance being washed out with a jet of hot 95 per cent. redistilled alcohol. The alcoholic filtrate is set aside to be added later to the other portions of the extract.

The well-drained material in the extraction cup is covered with fat-free cotton or filter-paper, placed in a continuous extractor and extracted 4 to 12 hours with 95 per cent. alcohol. The alcoholic extraction is followed with an ether extraction for a period of 1–12 hours. Following the ether extraction the sample is freed of ether and powdered as finely as possible in an agate or glass mortar or small coffee mill. The powdered material is then carefully collected and replaced in the apparatus and extracted further for a period of 12 to 24 hours with redistilled 95 per cent. alcohol. The several alcohol and ether extracts including the original alcoholic preserving filtrate are now combined. In the case of the ether extract most of the solvent is removed by evaporation and the residue transferred to the alcoholic portions with aid of hot alcohol followed by a little hot water. Any solid matter adhering to the flask after this treatment may be removed with the aid of a little chloroform and finally hot water. The combined extracts are then evaporated to small bulk as rapidly as possible, without overheating, on the steam bath, quantitatively transferred to an Erlenmeyer flask, and the evaporation continued until all of the liquid is driven off. Evaporation should be hastened by the use of a suction tube extending into the mouth of the flask and by adding absolute alcohol from time to time. The residue is finally dried over calcium chloride in a vacuum desiccator. At frequent intervals the desiccator should be filled with dry air and again exhausted.

The transfer to the Erlenmeyer flask is performed to facilitate subsequent extraction with ether of the dry residue. This extraction may be carried out by adding successive quantities of anhydrous ether to the residue, heating just to boiling on an electric hot-plate with stirring, and quickly filtering the separate portions of ether through an ashless filter-paper into a weighed flask. The filtered extract is evaporated to dryness

on the steam bath and the residue dried to constant weight at the temperature of boiling water as in the "Official method." From the weight of the residue so obtained the percentage of "crude lipides" in the original sample may be calculated. A moisture determination must be made on a duplicate sample of the original material if a dry weight calculation is desired.

Abstract of method, second procedure.—The sample is preserved in 80 per cent. alcohol as described under the first procedure. The insoluble residue, after separation from the alcoholic preserving liquid, is successively extracted with alcohol, ether, water and again with alcohol. The combined extracts are evaporated and the residue made into an emulsion with water. The aqueous emulsion is treated with chloroform and dilute hydrochloric acid. The mixture is well shaken and allowed to remain in a cool place until the aqueous portion becomes clear. This is drawn off through a filter. The lipide portion remains dissolved in the chloroform layer or as insoluble matter adhering to the walls of the flask and to the filter-paper. These three separations are brought together with aid of hot alcohol and the entire volume of liquid evaporated to dryness in a weighed flask, or made up to definite volume with hot alcohol after the evaporation of the chloroform, and aliquot portions taken for dry weight determination.

Details of operation.—Following the ether extraction as described under the first procedure, the insoluble residue is finely ground in an agate or a glass mortar or coffee mill. The powdered material is transferred to a flask and heated on the steam bath with about 100 cc. of water. Sufficient 95 per cent. alcohol is then added to bring the final concentration of alcohol to about 85 per cent. and the heating continued for another period. The mixture is then filtered through paper extraction thimbles, the residue being quantitatively transferred from the flask to the thimbles and extracted continuously with fresh 95 per cent. alcohol for 12 to 24 hours. The united alcohol, ether and water extracts are then evaporated on the steam bath at 75° to a syrupy consistency.[8] The residue is taken up with water and the aqueous emulsion is transferred to a glass-stoppered mixing cylinder or a volumetric flask having a capacity of one liter for each 100 grams of the sample. To wash adhering substances from the evaporation dish, small portions of chloroform are used which are added to the emulsion. The total volume of these washings should not amount to more than 20 or 25 cc. The flask or cylinder containing the emulsion is then filled to the mark with aqueous hydrochloric acid of such strength that the final volume

[8] Certain modifications are necessary from this point on if it is the intention to make determinations other than lipides on the same material, as outlined in the original method. For these consult Culpepper, Foster and Caldwell (13).

of liquid will contain 2 or 3 cc. of concentrated acid for each 250 cc. The mixture is vigorously shaken at frequent intervals for 2 hours, then placed in an ice-box for 24 hours or until the aqueous layer has separated and become quite clear. This is drawn off by gentle suction through a long tube of small bore. By exercising great care, the aqueous solution can be separated almost completely from the chloroform layer. Should any solid matter be drawn off, it should be collected by filtering through a dry ashless filter-paper and washed with a little water. The lipide matter will be found in the chloroform layer and adhering to the walls of the precipitation flask and to the filter-paper. The tip of the latter is perforated and any solid matter adhering to the paper washed into the precipitation flask with a jet of hot 95 per cent. alcohol. The contents of the flask are then quantitatively transferred to a weighed flash and evaporated to dryness on the steam bath. The residue is dried to constant weight at the temperature of boiling water, or else at room temperature in a vacuum desiccator over calcium chloride. This represents the total quantity of "crude lipides." (The method of determining the lipide fraction by separating the aqueous layer from the chloroform fraction as described is not applicable to all materials. In such cases it is necessary to follow the procedure indicated in Koch's student outline for the analysis of tissues to which reference has previously been made). In many cases it is desirable to determine in addition, the lipide phosphorus, lipide nitrogen and other constituents. For this purpose, the residue is dissolved in hot 95 per cent. alcohol and transferred to the volumetric flask. Ordinary redistilled alcohol is then added until the flask is about half full and then absolute alcohol to about two thirds full. The mixture is heated with gentle agitation on the steam bath until solution is uniform throughout, when the flask is filled to the mark with hot 95 per cent. alcohol. Aliquot portions are collected immediately for the various determinations, care being taken that the pipette for removing the liquid is first heated to the proper temperature in an oven or over a water-bath. Another modification which may be considered as a means of separating the lipide material from the other active ingredients is that of shaking the water emulsion of the alcohol-ether soluble material with pure ether at least four times or until a fresh ether extract no longer contains lipide material. The combined ether extracts are then shaken with distilled water to remove traces of water-soluble constituents. The ether extract is finally evaporated to dryness and the residue brought to constant weight as previously indicated.

General remarks.—The fact that the modified Koch method makes use of fresh instead of dried material is the chief point in its favor. If the sample is heated immediately with alcohol as described there is very little

likelihood of autolytic changes. Partial oxidation of certain unsaturated compounds may go on to a slight degree even with hot alcohol treatment, but the error from this source is probably much less than in any method where it is necessary to dehydrate the sample by exposure to heat and air.

Another distinct advantage of the method is the provisions which are made for the systematic extraction and separation into definite fractions for the determination of other substances, in addition to the lipides, from the same material. These determinations have been discussed elsewhere.

The use of both alcohol and ether as the first stage in the removal of lipides is perhaps preferable to the use of ether alone, since it is generally recognized that extraction with ether, no matter how prolonged, fails to completely separate all of these substances, especially the phospholipides, from the other tissue constituents such as the carbohydrates and proteins. The incomplete extraction is particularly evident with material high in starch. LEATHES (**43**) states in referring to the estimation of fats that "the use of the alcohol for the first stage of the extraction has the advantage of removing water, and leaving the tissue in a condition to give up its fat more completely to ether; but, besides this, alcohol seems effectually to liberate fat from cells in a way not clearly understood, possibly counteracting the tendency for some fatty substances to adhere to surfaces in the presence of water, much as alcohol will remove a dye that has adhered to charcoal suspended in water." One objection to the use of alcohol is the fact that it simultaneously removes along with the lipides most of the soluble carbohydrates which, unfortunately, interfere to a certain extent with the subsequent direct extraction of the syrupy residue with ether. This difficulty is not encountered in the second procedure in which the fatty compounds are emulsified and then separated from other constituents with chloroform and dilute hydrochloric acid. The use of dilute hydrochloric acid at this stage does not appear to have any hydrolytic effect on the lipides (**33**), but the whole second procedure is rather lengthy and bothersome.

3. KUMAGAWA-SUTO METHOD

ABSTRACT OF METHOD.—The fresh or dried plant material, or an alcoholic extract thereof, is saponified several hours with caustic soda solution. The mixture is then neutralized with acid, cooled, and thoroughly shaken out with ether to remove the fatty acids formed. The filtered ether extract is evaporated and the residue again taken up in absolute ether. The dry residue from this extract is finally dissolved in petroleum ether, the solvent evaporated, and the residue dried to consistent weight at 50° in a vacuum, cooled and weighed. This represents total crude fatty acids. A determination of unsaponifiable matter is made on this residue and the weight so

obtained subtracted from the total crude fatty acids, which gives the weight of true fatty acids. This figure multiplied by the factor 1.046 gives the approximate weight of the corresponding neutral glycerides.

Details of operation.—Either of two procedures may be followed in the method, (a) direct saponification of the material, and (b) alcoholic extraction with subsequent saponification of the alcohol extract. For direct saponification the material may be either fresh or in powder form. A sample of fresh material, weighing from 5 to 20 grams, is treated with 7 to 8 cc. of strong sodium hydroxide solution (sp. gr. = 1.5). If 5 grams are used, an additional 14 cc. of water are added. With 10 grams, 10 cc. of water are added, while above 20 grams it is unnecessary to make any further additions of water, the quantity of moisture present in the original tissues being sufficient. With dried material, 2 to 5 grams are treated with 25 cc. 5 N. sodium hydroxide solution (20 grams per 100 cc. of water).

The material and alkali in a 150 to 200 cc. beaker are placed on a boiling water-bath and covered with an open bell jar. At the end of 2 hours the warm mixture is quantitatively transferred to a 250 cc. separatory funnel. The last traces are removed from the beaker with 2 or 3 washings with small quantities of warm water. To the mixture in the separatory funnel, 30 cc. of a 20 per cent. hydrochloric acid solution (sp. gr. = 1.1) are slowly added, with cooling. When thoroughly cool, the acid liquid is vigorously shaken with successive portions of ether. For the first shaking, 70 to 100 cc. of ether are used. The second and third require only 5 to 10 cc. for each shaking. The aqueous layer is drawn off each time and the ether layer collected in a beaker. During the foregoing shaking operations, a precipitate forms in the funnel at the point of partition of the two layers. This precipitate is dissolved in 5 cc. of N. sodium hydroxide solution. The alkaline solution is shaken with 30 to 50 cc. of ether. Then the first aqueous acid liquid is added and the whole vigorously shaken. The separated ether layer is added to the other ether portion and the united extracts evaporated. The dry residue is then redissolved in absolute ether. The ethereal solution is filtered through a specially constructed asbestos filter (**39**) and evaporated to dryness. The residue is dried at 50° for 1 to 2 hours. Drying longer than this period should be carried out in a vacuum. The residue contains, besides fatty acids, also hydrocarbons, sterols, coloring matters and other substances. Some of these impurities are removed at this stage. To the residue while still warm 30 to 40 cc. of petroleum ether are added and gently rotated. The beaker is then covered with a watch glass and set aside ½ to 1 hour to permit the settling of resinous matter. The fatty acid solution is filtered through asbestos which is well washed with petroleum ether. The filtrate and washings are collected in a weighed

100 cc. beaker and evaporated. The residue is dried to constant weight at 50°, preferably *in vacuo*. This weight represents crude fatty acids.

It is particularly important that care be taken to sufficiently dry the residue from the ether extract before extraction with petroleum ether, in order to obtain the fatty acids in pure colorless form. The ether used for the last extraction, as well as the petroleum ether, should be pure and anhydrous. The time for drying the residue at 50° must not exceed 1 to 2 hours unless the drying is carried out under reduced pressure.

With many plant materials, especially those rich in starch and other polysaccharides, it is necessary first to make an alcoholic extract (b) before proceeding with the saponification of the lipides. The sample is extracted with absolute alcohol in the KUMAGAWA and SUTO hot extractor (39) until all but traces of lipides have been removed. After the addition of 7 to 8 cc. of a strong sodium hydroxide solution (sp. gr. = 1.5) to the alcoholic extract, the mixture is boiled under a reflux condenser for an hour. The alcohol is then allowed to evaporate, the saponification continuing meanwhile. When the alcohol has been removed, the residue is taken up with a little warm water and quantitatively transferred to a separatory funnel. The fatty acids are separated as described under the direct saponification procedure (a).

The residue of the original material which has been extracted with alcohol may still retain a small quantity of unextracted lipides, but in most cases this is too small to be significant. It may be recovered, however, by treating the residue essentially according to the direct saponification procedure (a), the only modification being that after neutralization of the saponification mixture the starch is hydrolyzed with strong acid to liberate any retained lipides.

To separate unsaponifiable matter from the final residue obtained as described in either method, the residue is redissolved in petroleum ether and introduced into a separatory funnel. To dissolve completely the residue and to transfer the solution quantitatively from the beaker to funnel requires 50 to 70 cc. of petroleum ether. Thirty to 40 volumes of N/5 nearly absolute alcoholic potash are added to the solution in the funnel which is then well shaken once. A clear solution results. A quantity of water is added to this equal to the volume of alcoholic potash used, whereby a separation occurs with the 50 per cent. alcoholic liquid as the bottom layer and the petroleum ether solution as the top layer. The unsaponifiable substances remain in the top layer, while the soaps go to the dilute alcoholic layer. After separation of the solution, the dilute alcoholic layer is again extracted with 30 to 50 cc. of new petroleum ether. After evaporation of the petroleum ether from the combined shakings the residue is freed from the small traces of fatty acids which it contains. This separation is accom-

plished by dissolving the residue in a little absolute alcohol, adding 0.5 to 1.0 cc. N/10 nearly absolute alcoholic soda, evaporating on the water-bath, drying the residue 15 to 30 minutes at 100° and then extracting with hot petroleum ether. The petroleum ether extract is filtered through asbestos into a weighed flask, evaporated and dried to constant weight at 100°. This weight represents total unsaponifiable matter. It must be subtracted from the weight of total crude fatty acids previously obtained to find the quantity of true fatty acids. The weight of true fatty acids multiplied by the factor 1.046 gives the weight of the neutral glycerides.

The above-described method of KUMAGAWA and SUTO has been modified by LEMLAND (**45**). Very briefly, the LEMLAND procedure consists in extracting the material 8 hours in the KUMAGAWA and SUTO extractor with alcohol, evaporating and drying to constant weight *in vacuo,* saponifying the residue, extracting and weighing the unsaponifiable matter, acidifying and extracting the residual soap solution and drying to constant weight and weighing the fatty acids. According to TERROINE, LEPAGE, VÉCHOT and WOLFF (**86**) this method is directly applicable to vegetable products provided they have been finely ground before extraction.

GENERAL REMARKS.—To convert numbers representing the weight of fatty acids into numbers representing the weight of neutral fat, KUMAGAWA and SUTO employ the factor 1.046. This factor is obtained by assuming that on saponification an equal quantity of each of the three fatty acids, oleic, stearic and palmitic acids, is obtained from corresponding triglycerides. The following, which is taken from KUMAGAWA and SUTO (**37**) indicates how the factor was obtained:

Molecular weight of triolein	884.8
Molecular weight of tristearin	890.9
Molecular weight of tripalmitin	806.8
average	= 860.8
	(oleopalmitostearate)
Molecular weight of oleic acid	282.3
Molecular weight of stearic acid	284.3
Molecular weight of palmitic acid	256.3
total	= 822.9
	(oleopalmitostearic acid)

$$\frac{\text{Oleopalmitostearate}}{\text{Oleopalmitostearic acid}} = \frac{860.8}{822.9} = 1.046 = \text{factor}$$

Strictly speaking, not all of the insoluble fatty acids, as determined by saponification, are derived from simple triglycerides, nor are the quantities of these fatty acids always present in equal proportions. Furthermore,

fatty acids other than the three mentioned may occur in the extract. In many cases an appreciable percentage of the total fatty acids may be obtained by hydrolysis of waxes and phospholipides which contain different percentages of fatty acids than do the simple glycerides or fats. These are sources of errors which probably cannot be overcome since it is impossible to know definitely the relative proportions of different classes of lipides giving rise to fatty acids on saponification.

In this method, the volatile fatty acids are lost and only the high-molecular fatty acids are determined. KUMAGAWA and SUTO point out that in the saponification method of LIEBERMANN and SZÉKELY much too great a quantity of low fatty acids are determined, and furthermore about 9 per cent. of the high-molecular acids escape determination.

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A STUDY OF CERTAIN CONSTITUENTS OF THE LEAF AND THEIR RELATION TO THE BURNING QUALITIES OF TOBACCO[1,2]

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Introduction

The term "burning qualities" as applied to smoking tobacco, usually includes several different elements. Of these, according to GARNER (**9**, p. 8), the fire holding capacity, the evenness and completeness of combustion, and the character of the ash are of most importance. A knowledge of the factors influencing the burning qualities of cigar-leaf tobacco is of considerable importance, both from the standpoint of the producer and the manufacturer, as the value of tobacco for cigar manufacture is based almost wholly upon these qualities. For this reason, therefore, considerable investigational work has been conducted upon this subject, and, as a result, it is now known that several factors, especially those of a chemical nature, influence the "burn" to a considerable extent.

Review of previous investigations

SCHLÖSING (**23**) was one of the first investigators to study the chemical factors affecting the burning qualities of tobacco. He treated tobacco leaves with various salt solutions and found that potassium salts of various organic acids were decidedly beneficial. He attributed their effects to the tendency of these salts to swell on heating, thus exposing more surface of combustible material.

By impregnating filter-paper with solutions of various salts, NESSLER (**20**, p. 73) found that the sulphate and carbonate of potassium increased the fire-holding capacity to a marked degree. He also observed that potassium acetate promoted combustibility in a manner similar to the carbonate, although this salt does not possess the power to swell when heated, at least to a marked degree, therefore, SCHLÖSING's interpretation of the catalytic effect of potassium salts of organic acids was probably not correct.

In analyzing the ash of good-burning tobacco, VAN BEMMELEN (**24**) always found a greater quantity of potassium present as the carbonate and lesser quantities as the chloride and sulphate.

[1] Published by permission of the Director of the Agricultural Experiment Station as scientific paper no. 433.

[2] This investigation was conducted in cooperation with Dr. W. W. GARNER, of the U. S. Bureau of Plant Industry, Office of Plant Nutrition and Tobacco Investigations, and Professor F. D. GARDNER, Department of Agronomy of the Pennsylvania State College.

MAYER (**16**) studied the influence of organic and inorganic substances on the capacity of filter-paper to glow or burn with a flame. The results obtained with inorganic compounds were similar to those obtained by NESSLER. In addition, he found that nearly all organic substances promote burning with a flame.

It has been suggested by BARTH (**2**), that the burn and characteristic glow of a good tobacco is due to a reduction of the alkali salts to oxides and small quantities of free metal, and that such a reduction would increase the efficiency of these metals as oxidizing catalysts.

BEHRENS (**4**), as a result of his investigational work on the burning qualities of tobacco, states that a high content of potassium in combination with citric, malic and oxalic acids largely determines the burning quality. GARNER (**9**, p. 19), is also of the opinion that potassium in such combinations is highly desirable, and further suggests that the favorable action of the carbonate obtained from the combustion of these substances may be due to its effect of functioning as a catalyst in combustion by taking up carbon dioxide and forming the acid carbonate at the most favorable moment and losing it later on. KRAYBILL (**12**), however, obtained results which seem to disprove this theory.

RIDGWAY (**22**) found a striking relationship existing between the fire-holding capacity and the degree of aggregation of various salts normally present in tobacco leaves. Under certain conditions of curing, it appears that there is a noticeable tendency for calcium and magnesium to crystallize as malates, citrates and oxalates. As these bases do not appear to promote the fire-holding capacity to any considerable degree, such a process is desirable, as relatively larger quantities of the tissues are thus left free to burn. He found that where such a crystallization did not take place, the burning quality was always poor.

According to LOEW (**13**, p. 38), the more oxidizable material, and the more oxidation going on within the tobacco leaf, the more will the crystals or "grain" develop in the curing and sweating processes and hence it will in many cases, although by no means in all, confirm the idea of some tobacco manufacturers that a well-developed grain is a good sign of the quality of the tobacco.

That chlorine exerts a deleterious effect on the burning quality of cigar-leaf tobacco is quite definitely established. MAYER (**14, 15, 16**), BEHRENS (**4, 5**), NESSLER (**17, 18, 19, 20**), FESCA (**6**), GARNER (**9**, p. 18), AMES and BOLTZ (**1**, p. 191), OLSON (**21**, p. 11), JENKINS (**11**, p. 95), and others have reported on the poor quality of cigar-leaf tobacco fertilized by chlorine-containing material. JENKINS (**10**) also states that while some chlorine is absolutely essential for the development of the tobacco plant, a large excess may prove deleterious to the burn.

Some work has been done to show a relation between the water-, ether-, and alcohol-soluble constituents of the leaf to its burning quality. GARNER (9, p. 18), made water extractions and found that the extracted leaf lost its glowing capacity. On examining the extract, he found that it contained the chloride, sulphate, nitrate, malate and citrate of potassium, ammonium and nicotine, and small quantities of lime and magnesium. Extracts from both good-burning and poor-burning leaves contained about the same quantities of potassium but the inferior leaf contained a much higher percentage of mineral acids. Hence he concluded that the potassium salts of organic acids such as malic and citric are the chief factors controlling the burn. Extraction with alcohol had little or no effect on the burn. It has also been shown by GARNER (9, p. 8) that some of the weaker organic acids, on the order of tannic, in combination with nicotine, are deleterious to the burning quality.

GRAHAM and CARR (7) made petroleum ether, sulphuric ether, alcohol and hot water extractions of tobacco and showed a relation between poor-burning quality and a high extractive content. They showed that tobacco fertilized with sodium nitrate or muriate of potash yielded a high amount of extractives.

Purpose and plan of the experiment

The object of this investigation was to make a study of the chemical composition of a given strain of cigar-leaf tobacco[3] in relation to its burning qualities, particularly as regards its fire-holding capacity. Particular attention was to be given to the determination of the quantity of ether-soluble organic acids present in cured and fermented samples, and also to their form of combination, as measured by the water-soluble and insoluble alkalinity of the ash. In addition, the effects of chemical treatments and climatic conditions were to be given due consideration.

MATERIALS USED

Eighteen plants, carefully selected from each of ten plots, 1/30th of an acre in area, located at Ephrata, Lancaster County, Pennsylvania, were harvested at the end of the growing season in 1925 and removed to the curing shed, where they were air cured. These samples were taken from the shed at the end of the curing season and dried under laboratory conditions. When they were thoroughly dry, the leaves, stems and stalks were separated, finely ground and transferred to air-tight containers and kept for analysis. This same procedure was followed in 1926.

The chemical treatment of the plots from which the samples were taken is shown in table I.

[3] HIBSHMAN strain, Pennsylvania Broadleaf.

TABLE I

FERTILIZER TREATMENTS OF THE EXPERIMENTAL PLOTS

OT	Treatments[a]										
	Manure	Sulphate of potash	Muriate of potash	Nitrate of potash	Manure salt	Bone flour	Precipitated bone phosphate	Acid phosphate	Urea	Ammonium nitrate	Cotton-seed meal
	tons	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
-1	10	....	200	....	....	250	....	....	......	......	400
-2	10	200	....	....	....	....	....	300	......	......	500
-5	10	....	....	....	....	....	....	....	......	......	....
-6	10	200	....	....	....	....	....	300	......	......	500
-9	10	....	....	....	400	250	....	....	......	......	400
-10	10	....	200	....	....	....	....	300	......	......	500
-1	10	....	200	....	....	....	....	300	65	......	....
2	10	....	....	214	....	....	120	....	......	......	....
3	10	200	....	....	....	....	....	300	65	......	....
6	10	200	....	....	....	....	....	300	......	85	....

[a] The potash, phosphoric acid and nitrogen-carrying materials, with the exception of manure and cottonseed meal constituted acre applications of
oximately 100 lbs. of potash, 48 lbs. of phosphoric acid and 38 lbs. of nitrogen.

Methods of analysis

In determining the total organic acid content of the different samples of tobacco, 10 grams of the material were transferred to a porcelain evaporating dish and 15 cc. of a 20 per cent. H_2SO_4 solution added. The mixture was then stirred thoroughly by means of a spatula, and finally enough powdered pumice stone was added to make the mass appear fairly dry. The mixture was then placed in a paper thimble and extracted with anhydrous ethyl ether for 24 hours. To the ether extract was added 200 cc. of water, the ether allowed to evaporate, the solution cooled, filtered and made up to a volume of 250 cc. Aliquots of 25 cc. were then taken and titrated with 0.04 N. NaOH, using phenolphthalein as an indicator.

In determining the crude ash and alkalinity of the ash, 2 grams of the sample were transferred to a platinum dish and carefully charred, the residue treated with water and filtered. The insoluble portion was placed again in the dish and ignited to constant weight. The filtrate was next added to the dish, evaporated, and the total residue carefully heated, cooled and weighed. The weight of the residue was calculated as crude ash.

About 150 cc. of hot water were next added to the crude ash and filtered. The residue on the filter-paper was washed well and the filtrate obtained was titrated with N/10 H_2SO_4, using methyl orange or xylene cyanal methyl orange as indicator.

The residue on the filter-paper was then placed in a platinum dish, treated with an excess of N/10 H_2SO_4, and the solution heated to boiling and filtered. The excess acid was then titrated with NaOH standard solution and the insoluble alkalinity was calculated accordingly. The usual methods of analysis were employed to determine the remaining constituents.

Experimental

Experiment I

Samples of the 1925 crop were taken and the more important constituents of the web determined. The results obtained are given in table II.

Experiment II

The total ether-soluble organic acid content of the stems, stalks and web portions of the cured samples, and also of the leaves of the fermented samples were determined. In addition, the total water-soluble and insoluble alkalinity of the web portions of the cured material were likewise determined.

The number of cc. of N. NaOH required to neutralize the ether-soluble organic acids in 100 grams of the air-dry material, and the number

TABLE II

THE PERCENTAGE COMPOSITION OF THE WEB OF CURED TOBACCO LEAVES OF THE 1925 CROP AS INFLUENCED BY FERTILIZER TREATMENT[a]

PLOT	CRUDE ASH	SiO_2	P_2O_5	CaO	MgO	SO_3	K_2O	Cl	N	NICOTINE
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
A- 1	24.08	2.10	0.50	7.88	1.25	1.46	1.89	1.12	4.70	4.89
A- 2	23.79	1.88	0.40	7.06	0.96	1.49	1.94	0.12	4.33	5.14
A- 5	23.99	2.52	0.40	7.30	1.24	1.40	1.93	0.19	4.38	4.61
A- 6	22.78	2.23	0.43	7.58	1.32	1.14	1.65	0.17	4.20	5.16
A- 9	24.15	2.14	0.44	7.93	1.21	1.27	1.95	2.29	4.37	4.56
A-10	23.10	2.23	0.44	7.64	1.15	1.35	1.97	0.95	4.63	4.79
B- 1	24.45	3.53	0.45	7.08	1.16	1.29	1.84	1.26	4.73	4.51
B- 2	23.32	2.09	0.48	7.83	1.31	1.61	1.97	0.19	4.60	4.82
B- 3	23.34	2.22	0.42	7.30	1.08	1.45	1.93	0.22	4.67	4.90
B- 6	23.59	3.87	0.43	7.17	1.14	1.28	2.03	0.25	4.87	4.09

[a] All percentages based on moisture-free material.

of cc. of N. H_2SO_4 required to neutralize the alkalinity of the ash of 100 grams of the same material are given in table IV.

EXPERIMENT III

Samples of the 1926 crop were taken after curing and determinations made of crude ash, chlorine, lime, magnesia and potash content of the web portions. In addition, the alkalinity and ether-soluble organic acid con-

TABLE III

THE CHEMICAL COMPOSITION OF THE 1926 CROP OF TOBACCO

PLOT	CRUDE ASH	Cl	K_2O	CaO	MgO	ALKALINITY OF ASH			ETHER-SOLUBLE ORGANIC ACID CONTENT
						Sol.	Insol.	Total	
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	cc.	cc.	cc.	cc.
A- 1	18.03	0.94	2.13	6.39	0.94	36	293	329	305
A- 2	22.85	0.17	2.29	6.43	0.89	48	283	331	281
A- 5	23.79	0.24	2.55	6.44	0.75	34	278	312	290
A- 6	21.08	0.17	2.38	6.29	0.86	51	323	374	290
A- 9	23.34	1.95	2.69	6.56	0.82	17	333	350	274
A-10	22.80	0.95	2.33	6.79	1.06	37	326	363	293
B- 1	22.78	1.07	2.04	6.83	1.09	25	296	321	286
B- 2	22.92	0.21	2.13	6.79	0.95	40	320	360	293
B- 3	22.54	0.19	3.37	6.75	0.88	39	306	345	283
B- 6	20.70	0.21	2.19	6.78	1.40	32	320	352	300

tent were also determined on the same samples. The results are presented in table III.

TABLE IV

THE TOTAL ETHER-SOLUBLE ORGANIC ACID CONTENT AND ALKALINITY OF THE 1925 CROP OF TOBACCO

Sample	Plant part	Cured material				Fermented material
		Alkalinity of ash			Ether-soluble organic acid content	Ether-soluble organic acid content
		Sol.	Insol.	Total		
		cc.	cc.	cc.	cc.	cc.
A– 1	Web	11	343	354	333	332
A– 1	Stem				234	
A– 1	Stalk				100	
A– 2	Web	39	322	361	336	
A– 2	Stem				300	
A– 2	Stalk				107	
A– 5	Web	22	332	354	347	351
A– 5	Stem				327	
A– 5	Stalk				139	
A– 6	Web	13	343	356	351	345
A– 6	Stem				304	345
A– 6	Stalk				128	
A– 9	Web	8	315	323	314	325
A– 9	Stem				167	
A– 9	Stalk				95	
A–10	Web	13	232	336	331	316
A–10	Stem				223	
A–10	Stalk				106	
B– 1	Web	14	317	331	313	305
B– 1	Stem				225	
B– 1	Stalk				103	
B– 2	Web	19	353	372	362	341
B– 2	Stem				311	
B– 2	Stalk				118	
B– 3	Web	35	309	344	345	324
B– 3	Stem				319	
B– 3	Stalk				163	
B– 6	Web	19	321	340	333	317
B– 6	Stem				324	
B– 6	Stalk				137	

Experiment IV

Samples of the web portions of the cured leaves of the 1925 and 1926 crops were taken and their hydrogen-ion concentration determined by the quinhydrone method (**3**). The results obtained are given in table V.

TABLE V

The hyrrogen-ion concentration of the 1925 and 1926 crops of tobacco

Year	Plots									
	A–1	A–2	A–5	A–6	A–9	A–10	B–1	B–2	B–3	B–6
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
1925	5.96	5.96	5.78	5.76	5.97	6.01	5.38	5.74	5.34	5.61
1926	5.95	6.19	6.08	6.16	6.10	6.20	6.37	6.78	6.08	6.27

Experiment V

Burning tests were conducted on strips of the fermented leaves of the 1925 and 1926 crops of tobacco. These strip tests of the fermented tobacco are shown in table VI.

TABLE VI

Strip tests[a] of the 1925 and 1926 crops of tobacco after fermentation

Plot	Burn 1925	Burn 1926
A– 1	Poor	Poor
A– 2	Fair	Fair to good
A– 5	Poor	Poor
A– 6	Good	Good to fine
A– 9	Poor	Poor
A–10	Poor	Poor
B– 1	Poor	Poor
B– 2	Good	Good to fine
B– 3	Fair to good	Good to fine
B– 6	Fair	Fair to good

[a] Definitions of terms in strip tests:

Very poor = tobacco does not hold fire. Coals badly.
Poor = tobacco holds fire but a few seconds. Coals badly.
Fair = tobacco holds fire over five seconds. Coals moderately.
Good = tobacco holds fire evenly over ten seconds. Coals slightly.
Fine = tobacco holds fire evenly until consumed. Coals very slightly.
Excellent = tobacco burns evenly until consumed without coaling and with light colored ash.

Weather conditions

Certain observations were made as to the weather conditions which prevailed during the growing seasons of 1925 and 1926. In 1925 the temperature was relatively high from May 31st until June 10th, and no precipitation occurred until June 25th. July was marked by severe electrical storms and heavy washing rains. The temperature rose gradually during the first two weeks of the month of August. No rain occurred from August 15th to September 13th. The plants were harvested shortly after this period.

The weather conditions for 1926 were quite different. It was moist and cool until June 10th, after which time the temperature rose to a relatively high degree and severe storms occurred during this hot period. The hot weather continued through July and August, but rain storms were quite frequent, breaking all previous rain records for August, and the excessive heat lasted until the plants were harvested. Following are the figures showing the actual precipitation during the growing season of 1926.

Month	Rainfall inches
June	4.31
July	4.57
August	9.12
September	4.47

No accurate measurement of the rainfall during the growing period was made in 1925, but, on the whole, the precipitation was much less than for the season of 1926.

Discussion of results

Experiment I, table II, shows that the chemical composition of the 1925 crop, from an elemental standpoint, was rather constant regardless of the fertilizer treatment. More chlorine, however, was found in the plants receiving chlorine-bearing compounds. The water-soluble and insoluble alkalinity of the ash also varied to a certain extent as shown by experiment II, table IV. The form of potash used appeared to play a part in causing this variation. Where the plants received muriate of potash, the water-soluble alkalinity was less as a rule than where the sulphate was used. A higher total alkalinity was obtained where potassium nitrate was applied. This table also shows a very wide potassium-calcium ratio, a wider ratio than is commonly found in good burning tobacco.

Analyses of the crop of 1926, according to experiment III, table III, show that approximately the same quantity of chlorine was present as in the 1925 crop. On the other hand, the lime and magnesia contents were lower and the potassium content was higher in the 1926 samples, but the potassium-calcium ratio was still too wide for satisfactory combustion. Where chlorine was present in relatively large quantities, a low water-soluble alkalinity of the ash was again apparent. On the whole, however, the water-soluble alkalinity of the 1926 crop was higher than that of the 1925 crop. The principal results obtained from these investigations show, with few exceptions, that there is a correlation between the water-soluble alkalinity of the samples and their burning qualities, and that this effect is more pronounced than that of the relative quantity of ether-soluble organic acids. It appears evident also that the form and amount of potash, and to a certain extent the season, have a great deal to do with the total quantity of water-soluble alkalinity present. If this is true, it is important to account for these effects.

Owing to the limited number of samples studied, it would not be proper to draw many definite conclusions, but it appears that these samples contain too little potassium and too great a quantity of calcium for the most desirable combustion. The data also indicate that the sulphate of potash is more desirable as a carrier of potassium than is the muriate. If potassium functions normally within the plant, it must act as a base. Combined with chlorine or sulphur it does not have the desirable property of neutralizing organic acids to any considerable extent. As chlorine is not normally required in any large quantities by the tobacco plant, it would probably tend to remain in combination with potassium, at least to a certain extent. On the other hand, sulphur may be utilized in relatively large quantities, and under these conditions more potassium would be rendered available for the neutralization of organic acids. As a matter of fact, however, analyses of the plants showed that the sulphur content was not increased by the addition of sulphates, so the reason for the difference in the basic properties of potassium from the sulphate, as compared with the muriate, must be sought elsewhere.

It is a well-known fact that the principal replaceable base of soil colloids, in a calcareous district such as Lancaster County, Pennsylvania, is calcium, and that the addition of potash salts to such a soil should lead to the replacement of calcium from such combinations and the formation of calcium salts. At the same time, it is possible to reverse this process. In other words, there is a competition existing between calcium and potassium, whereby either one or the other goes into colloidal combination. Under laboratory conditions, a sample of soil may be treated with muriate of pot-

ash and the replaced calcium may be completely removed by leaching, leaving potassium behind in basic form. On the other hand, it is quite possible to remove all absorbed potassium by the use of a solution of calcium chloride, leaving calcium behind in colloid combination and in basic form. Under natural conditions, neither of the above effects is ever observed, but a relatively dry year, such as in 1925, would have a different effect on the availability of potassium than would a comparatively wet year, as in 1926. Continual leaching of the soil would have the effect of rendering potassium available for absorption in basic form by the tobacco plants, while such a condition would not hold true under relatively dry conditions where soluble calcium salts tend to accumulate in the upper soil layers. Under the former conditions the tendency would be, in the light of our present knowledge, for potassium to assume the carbonate form. Under the latter condition, potassium would tend to assume the form of the muriate or sulphate, as the case may be. These reasons would tend to explain, in a measure, the beneficial effects of a wet season on the burning qualities of Lancaster County cigar-leaf tobacco, but would not necessarily hold true to any appreciable extent on soils lacking in colloidal material, unless the presence of relatively large quantities of calcium tends to inhibit the maximum absorption of potassium by plants.

The beneficial effect of sulphate of potash in a dry year, as compared with the muriate, may be due in part to the difference in chemical activity of the calcium compounds formed where these materials are added, the sulphate being more insoluble and less active chemically than the chloride, and not having the power of replacing potassium, which is possessed by the latter under ordinary conditions.

On the whole, the hydrogen-ion concentration of the 1926 crop was more nearly neutral than the 1925 crop. This would tend to have a desirable effect on the burn in an indirect manner, as it would more nearly approximate the optimum conditions for oxidase activity in the curing and fermentation processes.

Summary and conclusions

This investigation had for its purpose the study of the effect of certain constituents of the leaf in relation to the burning quality of cigar-leaf tobacco. As a result of these studies the following conclusions seem justified:

1. The ether-soluble organic acids of the plant appear to occur almost wholly in combination with the alkali and alkali earth metals, as measured by the water-soluble alkalinity and insoluble alkalinity of the ash respectively.
2. In practically all cases there is a parallelism between the burning qualities and the water-soluble alkalinity of the ash.

3. The season and the form of potash supplied as a fertilizer affected the water-soluble alkalinity of the ash in practically all cases.
4. The phenomenon of base exchange in the soil and the removal of certain active substances through leaching probably have considerable to do with the burn and composition of tobacco, but the difficulty involved in the absorption of potassium, under different conditions, in highly colloidal soils such as those of the experimental plots, should also be given consideration.
5. The lime content of the samples used in this experiment was too high and the potash content too low for maximum burning quality. Further work is in progress relative to increasing the potash content and lowering the calcium content of the tobacco grown in Lancaster County, Pennsylvania.

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MUCK-SOIL REACTION AS RELATED TO THE GROWTH OF CERTAIN LEAF VEGETABLES[1]

E. V. HARDENBURG

(WITH TWO FIGURES)

Soil reaction studies as related to the growth and character of crop plants have engaged the thought of numerous research workers during the past fifteen years. In fact, the reports of the majority of these studies bear dates more recent than the pioneer studies of HARTWELL and DAMON (**3**) which resulted in the classification of many economic plants according to their tolerance of soil acidity or their relative response to applications of lime. A review of the literature indicates that very few such studies with vegetables have been made and most of these have dealt with vegetables grown on mineral soils.

Review of literature

ARRHENIUS (**1**) determined the optimum pH for several crops, among which were sugar beets pH 7.5, radishes and rutabagas pH 7.0, peas pH 6, and turnips pH 4. He decided that most plants have two optimal points rather than a simple gradient. His method was colorimetric and may be criticized as giving, at best, only approximate results which obviously do not agree with more recent experiments. CRIST (**2**) grew Grand Rapids lettuce in nutrient water cultures of varying hydrogen-ion concentration and obtained maximum green weight at pH 5.0, with decreasing yields as the alkalinity increased from this point. Similarly, he grew lettuce in a soil culture of two-thirds acid muck and one-third coarse sand well fertilized with a 0-8-10 mixture and treated with hydrated lime in varying amounts. With each increase in lime application the green weight of lettuce and the ratio of top to root decreased. CRIST ascribed the injurious influence of the lime to change in permeability of the plant membrane, thus lowering its absorption of both nutrients and water, thereby causing limited malnutrition. LIPMAN, MCLEAN, and LINT (**5**), using sulphur in varying amounts on Sassafras sandy loam in a greenhouse experiment, obtained decreasing yields of barley, buckwheat and mustard, while radish yields were increased by the same treatments. MARTIN (**7**) applied sulphur flour in 300 pound per acre increments up to 1,200 pounds, to 1/60 to 1/40 acre

[1] Problem pursued in the Office of Horticulture, U. S. Department of Agriculture. Acknowledgment is made to Mr. J. H. BEATTIE, at whose suggestion and under whose advice the problem was undertaken, and to Dr. E. T. WHERRY, of the Bureau of Chemistry and Soils, for advice and assistance with the chemical phases.

plots of potatoes on three soil types and determined the hydrogen-ion concentration of the soil solution at frequent intervals from May 19th to August 16th. The acidity in all treatments increased up to June 24th, after which there was a gradual decrease. MARTIN explained this decrease in acidity by suggesting that all of the sulphur may have been oxidized by that time (71 days after application). Whereas the sulphur treatments decreased the pH value from 6.27 for the check plot to 5.10 for the plot receiving 1,200 pounds of uninoculated sulphur, the highest total yield was obtained from the last named plot. This would indicate an optimum pH value for potatoes under these conditions as low as 5.0. THERON (8) grew alfalfa, cotton, cucumbers, barley, and Bermuda grass in nutrient water cultures in which a wide range in pH values was set up and maintained by the addition of sulphuric acid and sodium hydroxide. Judging by the effects on both yield of green weight and appearance of the plants, he classified these crops according to their sensitivity to both H-ion and OH-ion concentration. Alfalfa and cucumber, showing a range of pH 4.8 to pH 6.0 for optimum growth and appearance, were classed as very sensitive, while barley and Bermuda grass having an optimum pH range of 4.5 to 7.0 and 4.5 to 8.0 were classed, respectively, as resistant and highly resistant.

ZIMMERLEY (10) has contributed what is perhaps the most detailed report on the relation of soil reaction to the growth of a given vegetable. He grew spinach, in a three-year experiment, in 15-inch glazed tile cylinders filled to a depth of 24 inches with each of three highly acid soils used in spinach production in the Norfolk area. Hydrated lime in amounts ranging from 1000 to 10,000 pounds to the acre was used to vary the pH values from 4.6 to 7.5. Yields of spinach increased in direct ratio to the amount of lime added up to pH 7.0 to 7.5 in 1924 and up to 6.5 to 6.9 in 1925 and 1926. Field test plots essentially substantiated the cylinder results by showing an optimum range of pH 6.5 to 7.0, depending on soil type and season. On these very acid soils, each 1000 pound increment of hydrated lime appreciably lowered the hydrogen-ion concentration up to the point of neutrality. Beyond pH 7.0, the increase in alkalinity was only pH 0.1 for each 1,000 pound application. In other unpublished work, ZIMMERLEY reports the optimum pH for lettuce grown on sandy soil as 5.6, growth at pH 4.7 being extremely poor. With beets, results were poor when the pH value went above 6.0. He furthermore reports danger of chlorosis in spinach with pH values greater than 7.0, instances of chlorosis being observed at pH values as low as 6.5.

The problem

Muck-land production of vegetables is not only increasing rapidly but there is an increasing interest in the number of kinds of vegetables which

may be adapted to soils of organic origin. Our markets are already well supplied with the more commonly produced muck crops from California, the middle west and localized areas in the east. The problem is now largely one of determining under what conditions other crops as well as these can be most efficiently produced. The subject of muck-soil reaction is by no means new but certainly too little is known of its basic character to permit of much confidence in our recommended cropping systems. The majority of our peat and muck soils now under cultivation are at least slightly acid, although many of them are classed as high in lime. So-called high-lime muck soils are obviously, not necessarily alkaline in reaction. A review of the literature indicates that many, perhaps most, of our common vegetables produce optimum yields on soils the pH value of which ranges between 5.0 and 6.5. There is great need for soil reaction studies of muck and peat soils with reference both to the influential factors and the response of the plant affected.

The primary purpose of the problem reported here was to study the performance of certain leafy vegetables when grown on a standard well-fertilized muck soil of varying hydrogen-ion concentration. Of secondary importance to the problem were such factors as comparative toxicity to these plants of active aluminum and sulphur under the conditions of the experiment, and absorption of aluminum, iron and calcium as determined by ash analysis of the oven-dried material from each culture treatment.

Materials and method

Lettuce, mustard, endive, and parsley were selected for study first, because they represent different degrees in sensitivity to soil acidity in the order named, as determined at the Rhode Island Station (**3**) and second because, being leafy vegetables, the yields could readily be compared in terms of oven-dry weights.

The soil used in all cultures was well oxidized muck from the Kankakee marsh area near South Bend, Indiana, the initial hydrogen-ion concentration several days after the fertilizer and manurial treatment being pH 5.0. The original soil as used in all checks and as maintained, except for the soil reaction treatments, analyzed as follows:

	Per cent.	
Moisture	53.47	
	Air-dry basis	Oven-dry basis
Ash	2.08	4.35
Phosphoric oxide, P_2O_5	0.18	0.38
Nitrogen, N	1.22	2.62
Lime, CaO	1.77	3.81
Potash, K_2O	0.13	0.28

These data indicate that the soil would class as low-lime muck having a comparatively narrow nitrogen-carbon ratio and, according to WHERRY (9), an active acidity of about 100 units (mediacid). In setting up the cultures, 10-inch clay flower pots were each filled with 15 pounds of thoroughly mixed and fertilized soil, the fertilizer treatment being 2000 pounds of a 2-8-10 mixture and 40 tons of clear cow-manure per acre. The fertilizer treatment was computed on the basis of 1,000,000 pounds of surface soil per acre and thoroughly mixed with the soil before potting. A uniform moisture condition was maintained by watering each culture to original weight twice a week throughout the growth period. After several preliminary trials with the soil reaction treatments to establish a fairly wide range in pH values, the soil was finally potted and the treatments applied on January 5th. The plant material used, together with dates of seeding, pricking-out, potting and harvesting are given in table I.

TABLE I

MATERIALS AND TREATMENT CALENDAR

CROP	VARIETY	SOURCE	SEEDED IN FLATS	PRICKED OUT	POTTED	HARVESTED
Lettuce	Grand Rapids	Bolgiano	11/22	12/6	1/10	3/29
Mustard	Giant Curled	Stokes	12/15	12/21	1/10	3/18
Endive	Green Curled	Stokes	11/22	12/21	1/10	4/6
Parsley	Giant Moss Curled	Stokes	11/2	11/22	1/10	4/15

Sufficient plants were grown to permit selection for size and uniformity. In general, the second pair of true leaves was developed at time of potting. All plants grew normally except no. 7 in the endive series (2000 pounds of sulphur) this remaining stunted and refusing to grow normally, although at no time was there evidence of disease or lack of chlorophyll development. A total of 32 plants of each kind was potted, 4 for each of the 8 soil reaction treatments. The original plan called for the use of aluminum sulphate to provide the higher hydrogen-ion concentrations. Preliminary tests indicated that even large applications would not result in pH greater than 5.0. Furthermore, some apprehension was felt that this material might result in injury from aluminum toxicity. Accordingly, commercial sulphur flour (uninoculated) was also used to provide the extremes of acidity in the series. Chemically pure calcium carbonate was used in varying amounts to provide the lower hydrogen-ion concentrations. Single plants were grown in each culture. Four untreated cultures were grown in each series to provide checks. This made a total of 128 cultures. All soil reaction treatments

were applied to the surface and thoroughly mixed with the upper 2 inches of soil, the aluminum sulphate being first dissolved in distilled water. These treatments consisted of 4000 and 2000 pounds of sulphur; 8000 and 2000 pounds of aluminum sulphate; check; 4000, 12,000, and 20,000 pounds of chemically pure calcium carbonate per acre, providing a range in pH values from approximately 4.0 to 7.0, respectively. The double wedge color comparator apparatus adapted and recommended by Dr. E. T. WHERRY, of the U. S. Bureau of Chemistry for soil extracts, was used in making the hydrogen-ion determinations. Readings were made on about one-half the cultures, including some from each series, at intervals of about 2 weeks throughout the growth period. The pH values given in the summary tables represent the average of these readings for each treatment. Before drawing the sample for each determination, a portion of the surface soil to an average depth of about 1 inch was mixed and the necessary quantity taken from this area.

TABLE II

RELATION OF HYDROGEN-ION CONCENTRATION TO GREEN WEIGHT OF TOPS IN LETTUCE, MUSTARD, ENDIVE, AND PARSLEY. (AVERAGE OF 4 PLANTS AND 5 TO 12 DETERMINTIONS FOR EACH TREATMENT)

TREATMENT	RANGE	LETTUCE	MUSTARD	ENDIVE	PARSLEY
Per acre	pH	gm. per plant	gm. per plant	gm. per plant	gm. per plant
4000 lbs. sulphur	3.95–4.05	375.00	213.43	344.13	111.95
2000 " sulphur	4.55–4.62	332.88	301.53	384.57	129.75
8000 " $Al_2(SO_4)_3$	4.68–4.76	410.88	318.38	366.80	111.75
2000 " $Al_2(SO_4)_3$	4.88–4.97	428.13	275.28	378.30	128.85
Check	5.00–5.04	423.75	274.63	473.75	145.50
2 tons $CaCO_3$	6.01–6.56	428.63	302.18	429.25	149.88
6 " $CaCO_3$	6.77–6.88	397.88	301.75	438.25	158.98
10 " $CaCO_3$	6.92–7.00	347.13	300.48	452.78	152.33

Reference to table II shows that even 10 ton applications of calcium carbonate failed to provide an alkaline soil reaction. Of the 16 cultures treated with the 10 ton application, there were several pH readings which exceeded 7.0 but in no case was the value greater than pH 7.1. The exceedingly high buffer effect here evident may be attributed to the high organic content and the assumed high colloidal complex common to this and similar muck soils. In this connection it should be noted that whereas the 2 ton lime application raised the pH value from approximately 5.0 to 6.5 or decreased the active acidity by nearly 100 units, increased amounts of lime affected the acidity very much less per unit application. The range in pH values shown in table II also indicate that the active acidity was much more affected by the

sulphur than by the aluminum sulphate per unit of application. The aluminum sulphate treatments not only did not result in apparent toxicity to any of the cultures but also were comparatively inefficient in increasing acidity of the soil solution.

The optimum pH range indicated for all four vegetables was obviously between 4.5 and 7.0. For lettuce the optimum range appears to be between pH 5.0 and 6.5. A typical series of Grand Rapids lettuce plants grown under the conditions of table II is shown in fig. 1. This may be considered

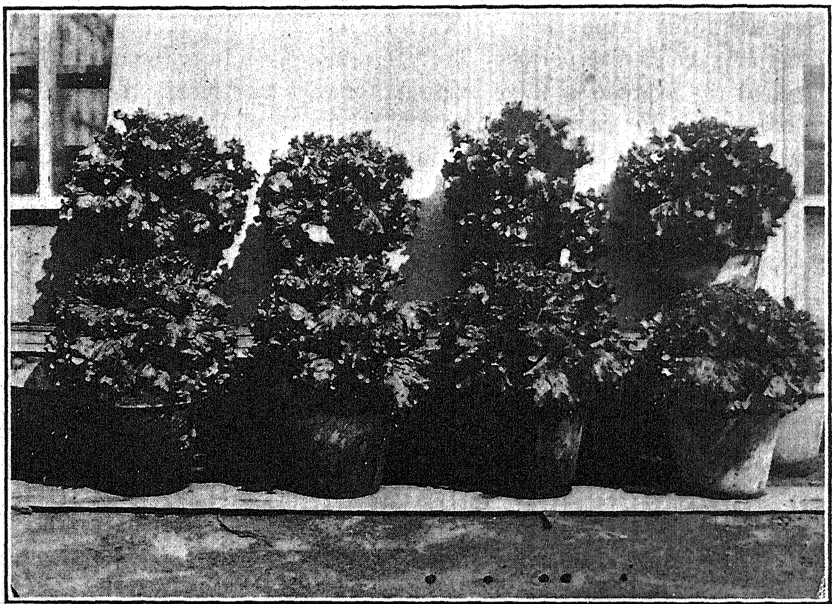

FIG. 1. Grand Rapids lettuce: A typical series from the muck soil treatments. Left to right: upper row, 4000 lbs. sulphur; 2000 lbs. sulphur; 8000 lbs. aluminum sulphate; 2000 lbs. aluminum sulphate. Lower row, check; 2 tons lime; 6 tons lime; 10 tons lime.

in fairly close agreement with the data of CRIST (2) whose optimum for this variety of lettuce was pH 5.0. In further agreement with CRIST are the data showing decreased green weight yields for the heavier lime applications. The lettuce yield was approximately as high at pH 4.0 to 4.5 as at pH 7.0. Further study of table II shows most consistent response to increasing alkalinity by the parsley, second endive, with results for mustard and lettuce favored by a reaction classing as mediacid to minimacid (WHERRY, 9). A typical series of the moss curled parsley is shown in fig. 2.

These results cannot be construed as confirming the classification of these vegetables as determined at Rhode Island (3) where, however, the soil used was a greenhouse potting soil of mineral origin.

FIG. 2. Moss Curled parsley: A typical series from the muck soil treatments. Left to right: upper row, 4000 lbs. sulphur; 2000 lbs. sulphur; 8000 lbs. aluminum sulphate; 2000 lbs. aluminum sulphate. Lower row, check; 2 tons lime; 6 tons lime; 10 tons lime.

Ash analysis of lettuce

Other workers in this field have made some study of the relation of soil reaction to ash content and ash analysis with particular reference to the absorption of calcium and aluminum under various treatments, CRIST (2), LINE (4), MAGISTAD (6), and perhaps many others not referred to in this discussion. Inasmuch as both lime and aluminum sulphate were used in this experiment, it was thought advisable to study their effect on the ash content of one of the crops grown. Lettuce was chosen because of its importance as a truck crop. The ash content of the oven-dried lettuce tops, together with the percentage composition and actual weight per plant of Al_2O_3, Fe_2O_3 and CaO are given in table III.

Obviously, there was no consistent relationship between hydrogen-ion concentration and either percentage of total ash or of aluminum-, iron-, and calcium-oxide absorbed. It is of some interest that even less aluminum was

TABLE III

ASH ANALYSIS[a] OF OVEN-DRY LETTUCE TOPS UNDER VARIOUS MUCK-SOIL TREATMENTS

TREATMENT	REACTION VALUE	ASH IN DRY MATTER		Al_2O_3		Fe_2O_3		CaO	
Per acre	pH	Per cent.	gm. per plant	Per cent.	gm. per plant	Per cent.	gm. per plant	Per cent.	gm. per plant
4000 lbs. sulphur	4.03	24.06	6.98	0.321	0.022	0.055	0.0038	2.27	0.158
2000 " sulphur	4.55	21.72	5.74	0.433	0.025	0.030	0.0017	2.06	0.118
8000 " $Al_2(SO_4)_3$	4.68	23.57	7.25	0.321	0.023	lost	lost	2.16	0.157
2000 " $Al_2(SO_4)_3$	4.92	24.36	7.81	0.413	0.032	0.050	0.0039	2.19	0.171
Check	5.00	22.80	8.03	0.280	0.022	0.035	0.0028	2.21	0.178
2 tons $CaCO_3$	6.38	27.75	9.76	0.334	0.033	0.080	0.0078	2.10	0.205
6 " $CaCO_3$	6.78	22.45	7.55	0.257	0.019	0.040	0.0030	2.08	0.157
10 " $CaCO_3$	7.00	21.94	6.48	0.284	0.018	0.037	0.0024	1.96	0.127
Average	....	23.58	7.45	0.330	0.024	0.047	0.0036	2.13	0.159

[a] Analyses by RUTH G. CAPEN, analyst, Crop Chemistry Laboratory, U. S. Bureau of Chemistry and Soils.

absorbed under the 8000-pound than under the 2000-pound per acre application of aluminum sulphate. The same is true of sulphur absorption from the two sulphur treatments.

Lack of any toxic effect of the aluminum sulphate treatments on any of the cultures can be explained on either of the theories set forth by LINE (**4**) or MAGISTAD (**6**). LINE's experiments indicated that aluminum will not remain soluble in soils less acid than pH 4.5, or than pH 3.5 if soluble phosphate is present. On the contrary, it will precipitate out as insoluble aluminum phosphate at pH concentrations higher than those indicated. MAGISTAD's work indicated that aluminum solubility does not increase rapidly until the acidity has reached at least pH 4.5.

With respect to the lime applications, there is at least some indication in table III that the absorption of aluminum, iron, and calcium decreased with each increment of lime added. These results accord with those obtained by CRIST (**2**) with Grand Rapids lettuce grown under similar conditions so far as aluminum and calcium are concerned. CRIST explained this differential absorption on the thoery that the "effect of applications of lime presumably on the permeability of the tissues was to reduce the intake of every mineral element for which the tissues were analyzed and the heavier the applications, the greater was the reduction, particularly as regards calcium and phosphorus." Unfortunately, the ash was in this case not analyzed for phosphorus. The final absolute water content of these plants decreased as the lime was increased, while the percentage content of water was approximately 92 for all three lime treatments. Here again the data are almost identical with those of CRIST.

Root and top ratios

Both tops and roots of all cultures were oven-dried to constant weight for oven-dry weights. These coincide very consistently with the green weights and, therefore, are not tabulated here. The roots were removed from the soil without washing. About two days intervened after the removal of the tops to allow the soil to dry further and the roots to shake out readily after inverting the pots. Considerable care was used to retrieve the more fibrous branch roots. Probably the best expression of root growth response to the various soil-reaction treatments is as per cent. of top weights. These data are given in table IV.

From the standpoint of average green weight of roots per culture treatment as related to soil reaction, the discussion given for top growth applies equally well. However, no very obvious correlation between hydrogen-ion concentration and root-and-top ratio appears in table IV, except for endive. It is evident here that root development in endive is consistently favored

TABLE IV

Relation of hydrogen-ion concentration to relative root and top growth in lettuce, mustard, endive, and parsley. (Average of 4 plants and 5 to 12 pH determinations for each treatment). (Green weight basis.)

Treatment	Reaction range	Lettuce	Mustard	Endive	Parsley
		Roots as percentage of top weight	Roots as percentage of top weight	Roots as percentage of top weight	Roots as percentage of top weight
Per acre	pH	Per cent.	Per cent.	Per cent.	Per cent.
4000 lbs. sulphur	3.95–4.05	10.26	11.57	13.84	48.28
2000 " sulphur	4.55–4.62	9.91	15.55	14.99	47.77
8000 " $Al_2(SO_4)_3$	4.68–4.76	9.50	15.27	15.29	59.06
2000 " $Al_2(SO_4)_3$	4.88–4.97	11.45	12.28	16.96	44.16
Check	5.00–5.04	9.99	20.12	15.94	46.29
2 tons $CaCO_3$	6.01–6.56	12.72	15.50	15.90	51.01
6 " $CaCO_3$	6.77–6.88	10.41	12.97	16.81	41.82
10 " $CaCO_3$	6.92–7.00	9.06	14.32	19.74	44.20

as the soil-reaction approaches neutrality. The optimum reaction for root development in lettuce was pH 6.38, for mustard pH 5.04, and for parsley pH 4.75. Whereas Crist (**2**) obtained a consistent increase in proportionate root development in lettuce with increased lime applications, somewhat opposite results are evident in table IV.

Water requirement

Record was kept of all water applied to the cultures throughout the growth period. It has been possible, therefore, to calculate the water requirement as affected by the various treatments. Since the harvest date, and therefore the length of growing season, for each vegetable varied by several days or weeks, no attempt was made to determine the absolute water requirement of each vegetable for purpose of comparison. Inasmuch as glazed pots were not used and no attempt made to prevent evaporation either from the surface of the soil or pots, the data on water requirement given in table V are calculated on the evaporation-transpiration basis.

Although there is no obvious relation between soil reaction and water requirement it may be noted that the maximum requirement for all four vegetables was reached in the more highly acid cultures. Of special interest is the fact that with lettuce, mustard, and parsley the water requirement increased markedly with each increase in lime application. The trend was opposite and less marked with endive. So far as the lime treated cultures

are concerned, these data coincide rather closely with the yields of dry matter and may be thus explained for all four vegetables.

TABLE V

RELATION OF SOIL TREATMENT TO WATER REQUIREMENT. (POUNDS OF WATER PER POUND OF DRY MATTER)

TREATMENT	LETTUCE	MUSTARD	ENDIVE	PARSLEY
Per acre	lbs.	lbs.	lbs.	lbs.
4000 lbs. sulphur	305.0	411.3	372.7	569.1
2000 " sulphur	352.6	283.0	478.3	630.7
8000 " $Al_2(SO_4)_3$	277.5	299.8	392.3	649.0
2000 " $Al_2(SO_4)_3$	286.3	329.7	345.5	568.3
Check	293.7	335.7	380.7	588.4
2 tons $CaCO_3$	261.1	252.0	342.0	512.7
6 " $CaCO_3$	285.6	273.1	338.2	527.6
10 " $CaCO_3$	348.3	283.1	328.6	538.6

Conclusions

The four leaf-vegetables selected for this experiment, because of their previous classification according to sensitivity to soil acidity, were grown in pot cultures on a highly fertilized low-lime muck soil having an initial pH value of 5.0. Sulphur, aluminum sulphate and calcium carbonate were applied in varying amounts to provide a soil reaction ranging from pH 4.0 to pH 7.0. Four cultures each of lettuce, mustard, endive, and parsley were given the eight soil treatments including the check. Due to the highly buffered nature of the muck, these treatments failed to provide as wide a range of hydrogen-ion concentration as had been hoped for. Even the 10 ton lime applications did not give a pH value greater than 7.0. Sulphur proved more active than aluminum sulphate as an acidifying agent. Contrary to expectation, no trace of aluminum toxicity was evident in any of the four vegetables from the 8000-pound application of aluminum sulphate. This is explained on the basis that the buffer influence of the muck soil prevented the development of a sufficiently high acidity to render the aluminum soluble in toxic quantity.

Best top weights of lettuce were obtained within a pH range of 5.0 to 6.5. The increased applications of lime to lettuce reduced the growth of both top and roots, decreased the total ash content, and the absorption of aluminum, iron, and calcium to a moderate extent. This influence is explained on the basis of change in permeability of the root tissues, thereby reducing the intake of the elements mentioned and probably of other

nutrients. Parsley and endive appear to respond to decreasing acidity more consistently than either lettuce or mustard.

No obvious correlation between hydrogen-ion concentration and root-and-top ratio exists for lettuce, mustard and parsley. Root development in endive appears to be favored by a culture medium which approaches neutrality.

The water requirement of lettuce, mustard, and parsley increased rather markedly with each increase in lime application. There was a corresponding, though much less marked decrease in the water requirement of endive for each increment of lime. With all four vegetables, the maximum water requirement was reached in the more acid cultures.

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THE EXTRACTION AND SEPARATION OF CHLOROPHYLL ($\alpha + \beta$) CAROTIN AND XANTHOPHYLL IN FRESH GREEN LEAVES, PRELIMINARY TO THEIR QUANTITATIVE DETERMINATION*

F. M. SCHERTZ

Introduction

This paper is published because of numerous requests which have been made of the author for information regarding the quantitative determination of the four chloroplast pigments in fresh green leaves. The method as described here is a modification of WILLSTÄTTER and STOLL'S method and has been developed and used in the Laboratory of Soil-Fertility Investigations, in the course of a series of investigations on the effect of nitrate, potash and phosphate on plant growth and production. It is hoped that the method as modified will prove highly valuable to those doing research in plant physiology as well as to those doing work along more practical lines. It is to be regretted that a satisfactory method for determining the two chlorophyll components separately is not available at the present time. Consequently, workers will have to be contented to know only the total amount of chlorophyll until more satisfactory methods than the ones described by WILLSTÄTTER and STOLL (5), are developed.

The method

The chief feature of the quantitative estimation of the pigments in fresh leaves is the thorough disintegration of the tissue and subsequent extraction with acetone containing only a low percentage of water.

The leaves may first be treated with aqueous acetone (30 per cent.) which removes yellowish plant acids, inhibits enzyme action, and at the same time removes none of the leaf pigments. The aqueous acetone extract is yellowish in color and appears to contain some of the yellow pigments,[1] but ethereal extractions and observations with the spectroscope revealed none of the yellow pigments in the 30 per cent. acetone extracts. This step is usually omitted by the author.

The details of the method are as follows: In a mortar, 25 cm. in diameter, are put 10 grams of fresh finely chopped or cut leaves[2] and a

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[1] The water soluble yellow pigments present here are flavones.

[2] The leaves should contain no petioles nor stems and should be representative of the plants studied. Practice has shown that if the leaves are dried, the pigments decompose more or less and consequently drying is never recommended.

little sodium carbonate to neutralize the acidity. The leaves are quickly mashed (5–30 min.) and ground thoroughly with 25 grams or more of washed quartz sand which serves to disintegrate the leaf substance. The leaves are first ground finely without the addition of any solvent. It is absolutely necessary here to grind till all of the tissue is thoroughly disintegrated if one expects the extraction to be quantitative. There are now added 50–100 cc. of pure acetone, and the material is ground again thoroughly, after which the whole is filtered through a filter-paper on a Buchner funnel 10 cm. in diameter. The leaf substance is then washed with pure acetone until the filtrate runs off colorless. Finally, wash by pouring 100 cc. or more of ether on the filter. The combined filtrate will be colored green and will contain carotin, xanthophyll, and both of the chlorophylls; in addition it contains as impurities, flavones and anthocyanins when they are present in the plant tissue which is being extracted. Complete extraction will require from 100–250 cc. of acetone and 75–150 cc. of ether. Add 50 cc. more of ether if necessary, for the larger the volume of ether, the easier it will be to wash out the acetone.

After the combined ether-acetone extract is obtained it is poured into a liter separatory funnel, and the acetone is then washed out by means of distilled water. First, wash by pouring the water through the ethereal acetone solution, or, better, by pouring the distilled water through a funnel. Add a small amount of water at first in the above manner, till a colorless or only slightly tinted yellow aqueous layer separates and then run off the aqueous layer. The yellow in the aqueous layer consists largely of flavones which may be tested for by adding alkali which causes the yellow color to deepen. Repeat this several times and then wash by gently rotating the liquids. If emulsions form they may be broken by washing with water containing sodium chloride.

In order to remove the remainder of the flavones, wash with a one per cent. solution of sodium carbonate. The flavones will come out as a deep yellow solution. If anthocyanins are present they also will be separated from the ethereal layer of carotin, xanthophyll and the chlorophylls, and will come out in the sodium carbonate wash water. The flavones and anthocyanins will be separated practically quantitatively while the chlorophylls and yellow pigments will remain in the ethereal layer. By this method a trace of the chlorophyll may be lost, but if the flavones and anthocyanins are not removed, the results when estimated colorimetrically would be far too high due to their presence. The addition of alkali later also greatly deepens the yellow color of the flavones which would tend to give too high results for the chlorophylls when estimated colorimetrically.

Tests made on a pure chlorophyll solution show that a one per cent. sodium carbonate solution will not remove any of the green pigments from an ether solution. Hence, any green pigments removed by the sodium carbonate solution are probably chlorophyll pigments which were already decomposed in the leaf.

The separatory funnel should not at any time be shaken because emulsions will form, the chlorophyll will go into the water in the colloidal state, and the separation will be more difficult. The wash water should remove none of the green or yellow pigments if the washing is properly done. The ether solution of the four pigments is now poured into a 250 cc. bottle. To this are added from 10–25 cc. of CH_3OH which has been saturated with KOH; this solution should have a clear color without a trace of yellow. It is shaken thoroughly and then set aside in the ice box till the next day when the chlorophyll will all be saponified and separated as a layer in the bottom of the bottle; the upper ethereal layer will contain the yellow pigments.

If it is necessary to proceed at once with the fractionation of the components, the saponification may be carried out by thoroughly shaking for 15 minutes or more. In this case there is danger that traces of the chlorophyll will not be saponified. The unsaponified portion will later be found in the xanthophyll fraction thus causing untrustworthy results to be obtained.

The alkaline solution of chlorophyllin salts, carotin and xanthophyll is now poured into a separatory funnel. Wash the bottle several times with 10–25 cc. of distilled water and add to the separatory funnel. Then wash the bottle with 25–50 cc. of ether to remove any yellow pigments. Add about 100 cc. or more of ether and shake strongly. Let stand 15 to 30 minutes and a yellowish ethereal layer of carotin and xanthophyll separates above, while the chlorophyllin salts separate as a greenish layer below. Run off the greenish layer and wash once or twice to remove any chlorophyllin salts which tend to remain in the ether layer with the yellow pigments; then add water to make about 75 cc. and extract the chlorophyllin solution with about 100 cc. of ether to remove traces of yellow pigments. Do this a second time if necessary, when the ether will be colorless or only slightly colored yellow. The greenish chlorophyllin salts are now run off into a 100 cc. volumetric flask. The ether solutions are combined and washed once with dilute potassium hydroxide solution (10–25 cc.) and twice with distilled water (10–25 cc.) to remove traces of alkali and chlorophyllin salts. These combined chlorophyllin solutions are made up to a volume of 100 cc. with water and the chlorophyllin salts are then estimated according to a method described in another paper (3). The

chlorophyllin solution may be made up to a larger volume if it is more convenient for the worker. Store the chlorophyllin solutions in an ice box till they are estimated.

The combined ether extracts are now evaporated in a flask at 50° C. or lower, to a few cc. and then evaporated strongly (using suction) to remove all traces of ether.

The residue is transferred by means of about 110 cc. of fresh petroleum ether and about 50 cc. of 85 per cent. methyl alcohol (made by making up 85 cc. of absolute CH_3OH to a volume of 100 cc. with water) to a separating funnel. Shake, let stand till the layers fully separate, and run off the methyl alcohol layer. From this petroleum ether solution the xanthophyll is further extracted by repeated shaking with methyl alcohol as follows: (1) two extractions with 25 cc. of 85 per cent.; (2) one extraction with 25 cc. of 90 per cent.; (3) two extractions with 92 per cent., using about 25 cc. for each extraction. Usually the last extraction is not colorless, and so further extractions with 92 per cent. methyl alcohol are made. Let the methyl alcohol and petroleum ether solution stand for about 5 minutes after each shaking for the layers to fully separate. In this way by means of the methyl alcohol the orange yellow xanthophyll is separated from the orange colored carotin which remains in the petroleum ether layer.

To the combined methyl alcohol xanthophyll extractions add 150–200 cc. or more of ether and pour the whole into a large separatory funnel. Now add distilled water equal in volume to the volume of methyl alcohol from which the xanthophyll is to be separated, shake vigorously and allow to stand about 15 minutes, when the xanthophyll will have collected in the upper ether layer, with a colorless aqueous methyl alcohol layer below. Treat, if necessary, with 10 cc. or more of a saturated solution of Na_2SO_4 or NaCl[3] to break emulsions, which, if not broken up, will make the separation of xanthophyll incomplete and very unsatisfactory. Run off the aqueous methyl alcohol solution and extract it a second time with ether. Now wash the combined ether solution once or twice with distilled water to remove traces of the salt and methyl alcohol. Run the ether solution of xanthophyll into a flask and evaporate at low temperature (using suction) to about 50 cc. Pour into a separatory funnel, add ether, and then a little water, to separate any methyl alcohol from the ether solution. Run off the lower layer, wash with water, and let stand a moment till most of the free water separates. Make up to 100 cc. in a volumetric flask and then estimate the pigment colorimetrically, or spectrophotometrically if very accurate results are desired (**2**).

[3] STECHE (4) recommends KCl and $CaCl_2$ for breaking up colloidal formations. These salts should be tried.

If the ether solution is not clear, it may be cleared by adding a few drops of absolute ethyl alcohol or by allowing it to stand for a few hours, by which time the water will settle out; or one may dry it by filtering through a layer of anhydrous sodium sulphate.

The petroleum ether solution of carotin is washed two or three times with distilled water to remove traces of methyl alcohol. The solution of carotin is then allowed to stand till the free water separates or is dried by filtering through anhydrous sodium sulphate and then run into a 100 cc. volumetric flask, cleared, made up to 100 cc. and is then estimated (**1**).

The following chemical changes take place in the method of separating and estimating the leaf pigments here outlined.

According to WILLSTÄTTER no chemical changes take place in the yellow pigments by the method of separation. During the separation of the yellow pigments, the two chlorophylls are changed when alkali is added as is shown in the following reactions:

$$\underset{\text{Chlorophyll } \alpha}{C_{31}H_{29}N_3Mg\ (NH.CO)\ (COOCH_3)\ (COOC_{20}H_{39})} + KOH \rightarrow \underset{\text{Chlorophyllin } \alpha}{C_{31}H_{29}N_3Mg\ (NH.CO)\ (COOH)\ (COOH)}$$

$$\underset{\text{Chlorophyll } \beta}{C_{32}H_{28}O_2N_4Mg\ (COOCH_3)\ (COOC_{20}H_{39})} + KOH \rightarrow \underset{\text{Chlorophyllin } \beta}{C_{32}H_{28}O_2N_4Mg\ (COOH)\ (COOH)}$$

Since working out the above method of determining chlorophyll and the yellow pigments in leaves a paper by STECHE (**4**) has come to the writer's attention. His recommendations are similar to those the writer has incorporated in the method as given in this paper. He omits the preliminary extraction because the flavones are later removed by washing from the ethereal solution of the chloroplast pigments. The writer has used a solution of sodium carbonate to remove the flavones and anthocyanins. He saponifies the chlorophyll in three steps using 5 cc. of saturated methyl alcoholic potash each time. His third change in WILLSTÄTTER's method concerns washing the acetone from the ethereal solution of chloroplast pigments. Instead of distilled water he uses a solution of sodium chloride, or, better, potassium chloride. He washes the ethereal solution of chloroplast pigments 5 times, each time with 100 cc. of 1 per cent. KCl solution, and then once with 100 cc. of 3 per cent. KCl solution. This step is similar to the writer's wherein NaCl solution is recommended. Any one interested in determining chlorophyll should read STECHE's paper.

Summary

1. A method is described for quantitatively extracting the chloroplast pigments from leaf tissues.

2. The chloroplast pigments after being freed from accompanying materials are then quantitatively separated into carotin, xanthophyll, and chlorophyll ($\alpha + \beta$).

3. These pigments are then quantitatively determined by methods published by the author.

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FURTHER EVIDENCE OF THE ESSENTIAL NATURE OF ZINC FOR THE GROWTH OF HIGHER GREEN PLANTS

ANNA L. SOMMER

(WITH THREE FIGURES)

Very little has been done to demonstrate the essential nature of zinc for the growth of higher green plants. Although MAZÉ, as early as 1914 (1), showed it to be essential for the normal development of maize, no further work seems to have appeared until the writer showed that zinc as well as boron (2) is essential for the normal growth of barley and sunflowers.

It is not evident that a given element which is essential in only small quantities for plant growth may be equally necessary for all groups of flowering plants. It is therefore desirable to extend the range of experimental material, which in the case of zinc has embraced only two representatives of the grasses and one of the Compositae. We now have plants of two other families to add to the list.

The technique used in this investigation was the same as that described in the paper reporting the work on barley and sunflowers. All salts were especially purified, the solution culture method was employed, and the plants grown in pyrex containers. Precautions were taken to prevent contamination of the solutions by dust.

The solutions had the following composition:

	Per liter		Per liter
KNO_3	0.80 gm.	Mn (as $MnSO_4$)	0.0015 gm.
KH_2PO_4	0.15 gm.	Al (as $Al_2(SO_4)_3$)	0.0005 gm.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	NaCl	0.0127 gm.
$CaSO_4$, saturated solution	300 cc.	Cu (as $CuSO_4$)	0.000125 gm.
B (as H_3BO_3)	0.0005 gm.	I (as KI)	0.00025 gm.
		F (as KF)	0.00025 gm.

0.5 p.p.m.

Iron as $FeSO_4$ was added when needed. An excess of silicon as SiO_2 was added to the solutions. The control cultures received in addition 0.0005 grams per liter of zinc as $ZnSO_4$.

To add to the families of plants already investigated, the Polygonaceae, represented by buckwheat, and the Leguminosae, represented by Windsor beans, *Vicia Faba,* and red kidney beans were studied.

Buckwheat responded to the lack of zinc in a way similar to that of the sunflowers; that is, the plants without zinc grew more slowly than the controls even during the early stages of development. None of the buckwheat plants, however, died before reaching the flowering stage. At maturity, plants grown without zinc were much smaller than the controls. The aver-

age dry weight per culture of buckwheat grown without zinc was 1.94 grams, that of the controls was 8.45 grams. Ten cultures of four plants each were included in each group. Buckwheat plants grown with and without zinc are shown in figure 1.

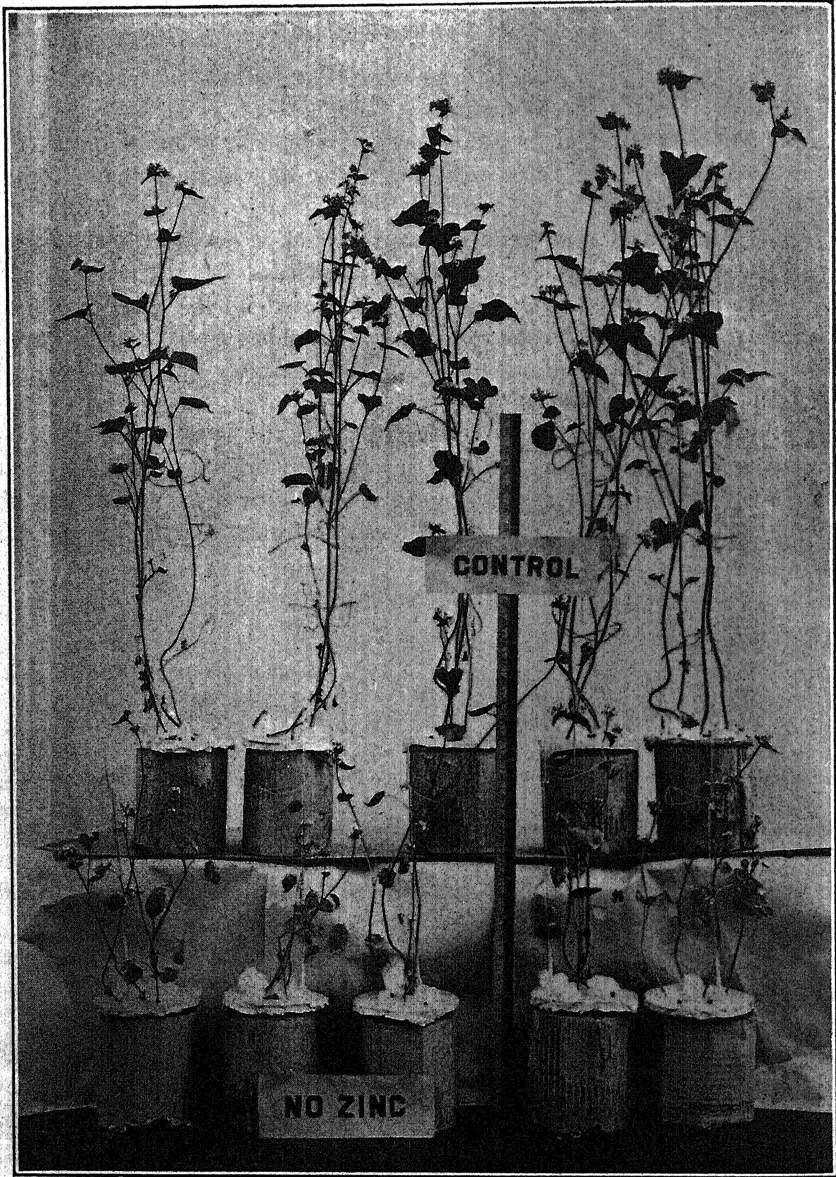

FIG. 1. Buckwheat grown with and without zinc.

The response of the legumes was quite different. In the case of the Windsor bean there was no visible difference between plants grown with and without zinc until the flowering stage was reached. At this stage there was a sudden and rapid abscission of the leaves. Most of the flower buds fell off and in the very few cases in which seed pods appeared, no seeds were formed. The average dry weight per culture of Windsor beans grown without zinc was 13.3 grams, that of cultures with zinc was 27.0 grams. The plants in culture solutions with zinc were growing well, blooming freely, and producing seeds when harvested. The plants without zinc were in very poor condition. Ten cultures of four plants each were included in each group. Windsor beans grown with and without zinc are shown in figure 2.

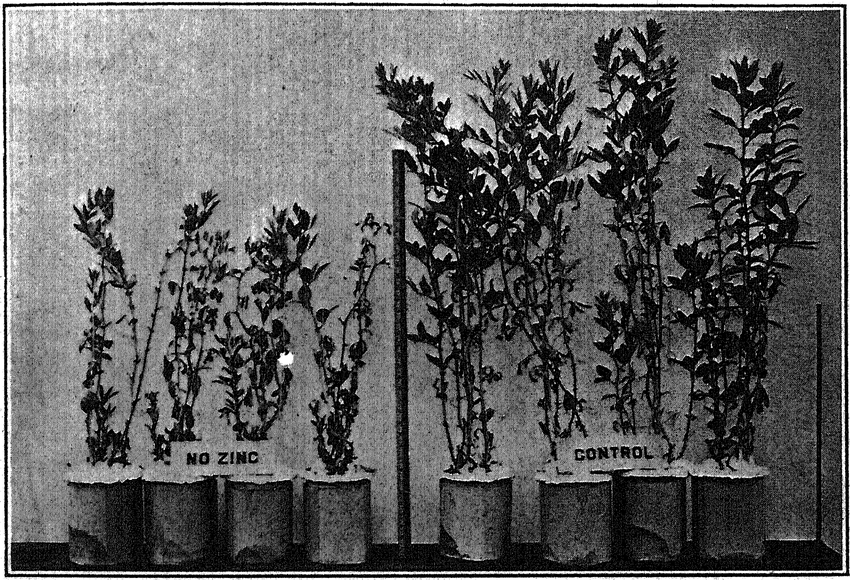

FIG. 2. Windsor beans grown with and without zinc.

The results obtained with red kidney beans were very similar to those obtained with the Windsor beans. The same number of cultures and plants per culture were employed. Red kidney beans grown with and without zinc are shown in figure 3.

The difference in the response of different plants to the absence of the same element is a very interesting and probably important study. In the case of zinc, the few plants studied by the writer presented three different types of response. In the case of wheat and barley, the plants appeared

normal for about two weeks, the tops then stopped growing and gradually dried up, all plants without zinc dying in the early stages of development. In the second type of response, that shown by sunflowers and buckwheat, plants showed the effects of the lack of zinc very soon after being transferred to zinc free solution. They made very poor growth but lived much longer than the barley, and, in the case of the buckwheat, produced a few poor seeds. The third type of response displayed by the legumes is interesting not only because the effects of the absence of zinc was not shown until the flowering stage was reached but also because of the apparent suddenness and the severity of the injury. The mature buckwheat plants and

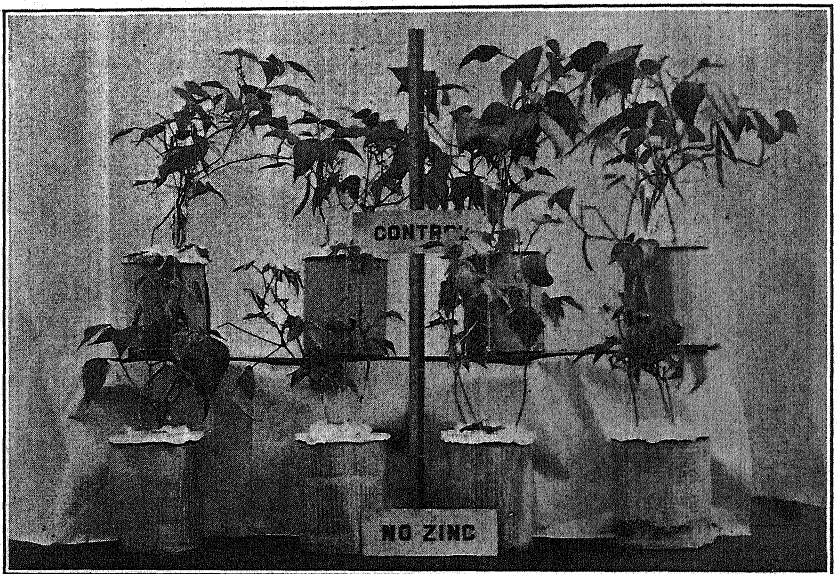

FIG. 3. Red kidney beans grown with and without zinc.

the sunflowers which survived gave the appearance of plants which had been grown in poor soil or with insufficient moisture while the legumes looked like badly diseased plants very soon after the first symptoms of injury from the lack of zinc appeared. It seems to the writer that these differences are too great to be accounted for by the difference in the amounts of zinc that may be stored in the seeds and that they indicate that there must be significant physiological differences among the higher green plants. Observations on plants grown with and without boron strengthen this conviction. Most of the fairly large number of dicotyledonous plants studied by the author make little or no growth in the absence of boron and,

where some growth is made, the roots are the first to show the injury. On the other hand several members of the grass family make considerable growth and the tops are the first to show injury.

Earlier work on essential elements did not show zinc to be necessary because of the lack of refinement of methods, the importance of which I have discussed in another paper (3). The fact that plants of five different families require zinc for normal growth makes it probable that this element is indispensable for all of the higher green plants. When we consider the work of mycologists who, under less carefully controlled conditions, have shown it to be so beneficial to certain of the fungi, we may expect that future investigations will place it in the list of elements essential to the whole of the vegetable kingdom.

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A STERILE CHARACTER IN SOYBEANS[1]

F. V. Owen

(WITH TWO FIGURES)

In 1924 a progeny of Manchu soybeans was found to be segregating for sterility. The segregation has since proved to be very clear-cut and a single factor mutation is held to be responsible for the results. Careful records had also been taken of the parental plants for two previous generations so it is quite certain that the origin was by means of a genuine mutation and not merely a heterozygous selection.

The following results were obtained by growing seed from the plant (No. 26–5–11) which bore the first sterile seeds.

	Normal plants	Sterile plants
Observed	83	28
Calculated	83.2	27.8

The segregation of the progeny happened to be almost perfectly in line with expectation. Approximately two-thirds of the normal segregates also proved to be heterozygous for the sterility factor in the next generation so there is little doubt about the mode of inheritance.

The exact cause of this sterility has not been determined but apparently the ovule is non-functional as well as the pollen grains. At first it was hoped that the sterility factor had effected a wholesale emasculation for convenience in hybridization, but all efforts to produce seeds on sterile plants have failed. Pollen grains are formed which appear to be normal until the anthers are about to dehisce, but at this time they take on a shrunken appearance which makes it very easy to classify normal and sterile plants microscopically. Heterozygous plants appeared perfectly normal in all respects.

The physiology of sterile plants

The behavior of the sterile plants after flowering is of special interest because growth in soybeans is largely determinate. After the first flowers drop from the sterile individuals there is frequently a second flowering and small pods are sometimes formed, but no ovules have ever developed and the sterility has been complete in every case.

These sterile plants are perfectly normal during the early stages of growth but after flowering time there are noticeable differences between normal and sterile plants. Sterile plants take on a much darker green

[1] From the Department of Genetics, paper no. 90 Wisconsin Agricultural Experiment Station. Published with the approval of the director of the station.

color, the leaves thicken, and the stems sometimes become greatly enlarged. Furthermore, the leaves on these plants remain green and firmly attached to the stem long after normal segregates have matured. Fig. 1 shows the characteristic form of a sterile plant, and fig. 2 the contrasting normal individual.

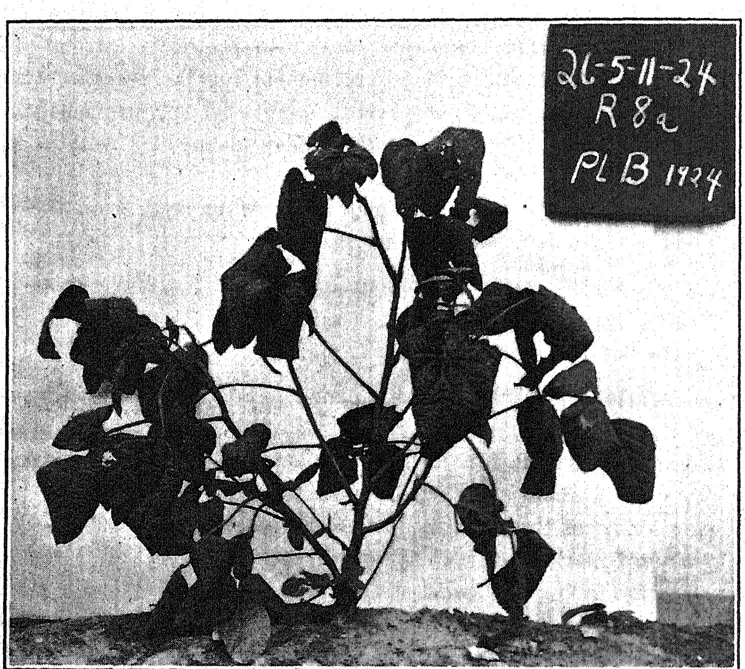

FIG. 1. Where can this plant store its soybeans? The chief significance of this sterile character lies in the nature of growth after flowering time. The plant still elaborates food material, but the determinate type of growth prevents the formation of new shoots. With storage channels all cut off, this "manufacturing establishment" becomes saturated with the very materials which it was designed to produce. There is a single factor difference between sterile and non-sterile plants.

No test of starch has been secured in the stems of normal plants after maturity but the iodine test has indicated the presence of considerable starch in the stems of sterile plants at the end of the season. This condition is to be expected because these sterile plants must be synthesizing considerable food material all the time, but the parenchymatous cells of the leaves and stem seem to constitute the only storehouse available. The whole situation, therefore, is quite like a modern canning factory that runs short of the usual tin cans. The section of the factory where raw materials are taken in may work ever so efficiently at first but it soon becomes congested from lack of storage containers.

The peculiar behavior of sterile plants, naturally deprived of a storehouse for elaborated food material, has also been of interest in connection with a study of mottling of the seed coat because of a possible connection between the two phenomena. In previous studies (**2**) abnormal plants have been described which resembled sterile plants very much in that the leaves thickened and turned a dark green color. These abnormal plants developed an extreme amount of mottling in the seed coat, and it is assumed that the excess accumulation of elaborated food material was associated with the development of glucosidal pigments.

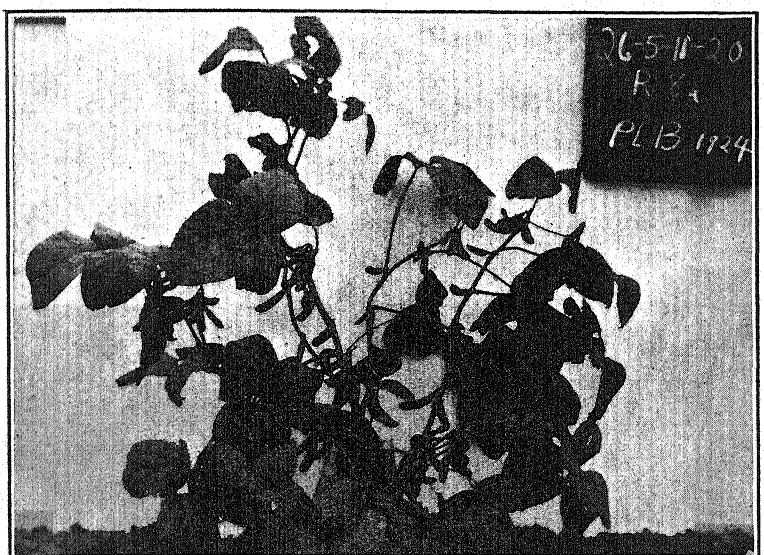

FIG. 2. Normal plant with plenty of pods for storage space. The seeds of normal plants make a natural storehouse for elaborated food material. It is believed that this is a very important factor in determining the ultimate efficiency of normal thrifty plants. This normal plant is a sib to the sterile individual shown in fig. 1.

It is hoped that a more thorough knowledge of the physiological peculiarities of sterile plants of this sort may sometime be available. MURNEEK'S (**1**) interpretation of the stimulus given to a plant by setting fruit is very interesting and it seems natural to suppose that a plant should be at its maximum metabolic efficiency after a full growth of foliage has become established, provided that good storage channels are available. It is likely that a more careful study of plants entirely deprived of reproductive organs might also be pursued with profit. The soybean should be particularly well adapted for such studies because of the determinate type of growth.

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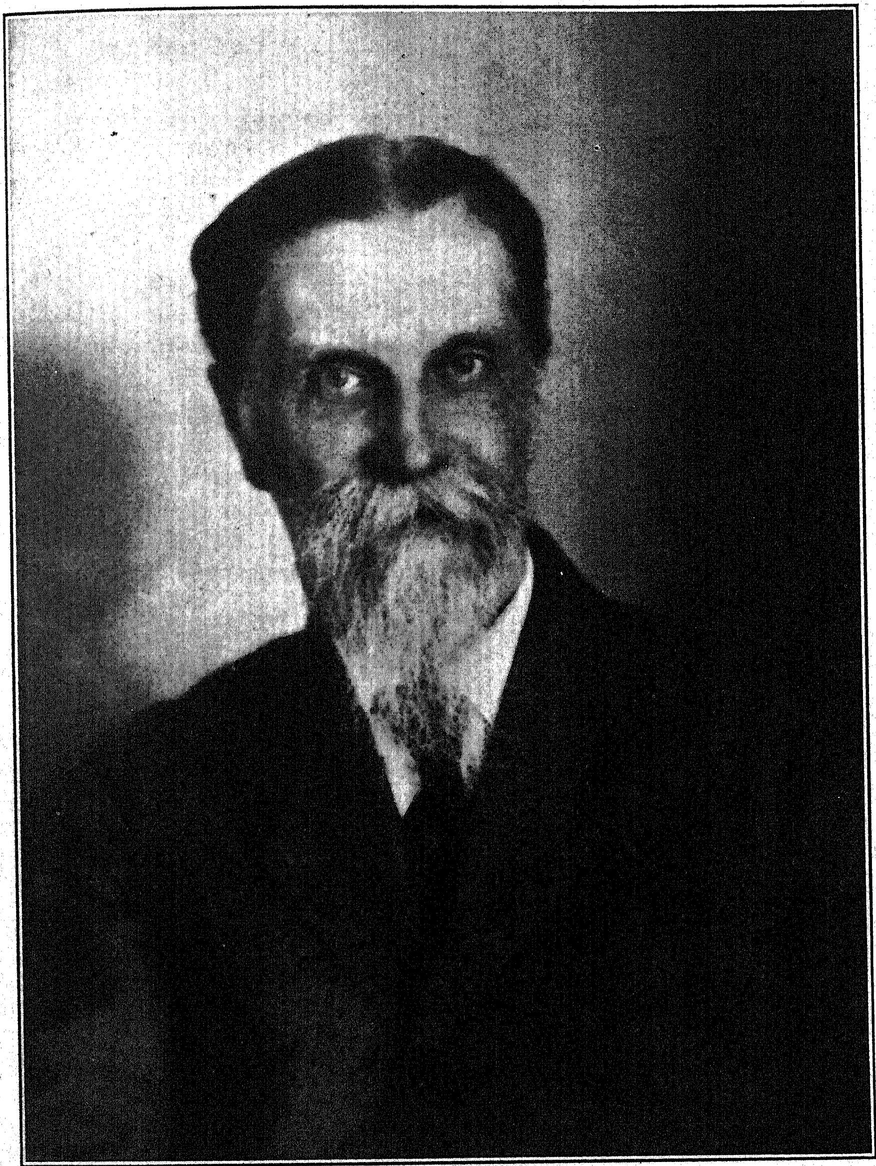

CLEMENT ARKADEVICH TIMIRIAZEFF

BRIEF PAPER

TIMIRIAZEFF AND THE TIMIRIAZEFF AGRICULTURAL INSTITUTE

(WITH ONE PLATE AND TWO FIGURES)

The devotion of CLEMENT ARKADEVICH TIMIRIAZEFF to the welfare of the Russian common peoples and his staunch advocacy of intellectual freedom in the universities of old Russia has placed him in the front rank of the leaders in the revival of new Russia. Much of the good in the new régime is the work of TIMIRIAZEFF and his associates, and he lived to see the overthrow of the Tsar's régime and the adoption of part of the ideas which he advocated, yet not to see the later evils of the revolution, since he died in 1920. Plate VI is from a portrait of the great plant physiologist.

Born on May 23, 1843, of an old and noble family at Leningrad TIMIRIAZEFF's life covered a period of active change of ideas in the biological sciences, and in this change he took an active part. He was responsible for the introduction of many of the newer ideas in biology and agriculture in Russia.

TIMIRIAZEFF's education at home, according to the Russian custom, equipped him with a thorough knowledge of languages and with a fundamental training for the University. He matriculated under the science faculty of the University of Leningrad in 1861. He passed his license examination five years later and was awarded a medal of gold for the excellence of his paper on the Liverworts. Following his first University degree he published a number of scientific papers and also took part in the political and literary developments of that day.

In 1868 appeared his first paper on photosynthesis. Most of the writings of TIMIRIAZEFF have been upon questions concerned with the chemistry and physics of carbon assimilation. He went into problems in the most minute detail, yet never lost sight of the fundamental principles underlying the research. It was this happy combination of minute exactness in detail and broad perspective of the whole of the research, which he instilled into his students with rare ability. TIMIRIAZEFF as a teacher had a remarkable faculty of imparting his great fund of scientific and historical information to his students as well as of creating in his class rooms an atmosphere of the highest culture. His lectures were characterized by clearness, and his demonstrations by simplicity.

TIMIRIAZEFF's advanced training was acquired at Heidelberg where he worked under BUNSEN, KIRCHOFF, and HOFMEISTER, and took a course under

HELMHOLTZ. Training in this exacting school left its impress upon all his experimentation. But it was at Paris that he received his inspiration under BOUSSINGAULT, and it was to BOUSSINGAULT that he frequently returned after his establishment as professor at Moscow. The connection between his experiments and the teachings of BOUSSINGAULT is not hard to trace. TIMIRIAZEFF published a memoir of BOUSSINGAULT in 1887. While still a student at Paris he heard BERTHELOT lecture on thermo-chemistry and frequently afterwards renewed his friendship on visits to Paris.

On his return from abroad he was elevated to the rank of professor by the Moscow Academy of Agriculture. He started at that institution courses in all the branches of botany. In 1877 he was appointed professor of anatomy and plant physiology at the University of Moscow.

TIMIRIAZEFF applied himself to the solution of the agronomic problems of Russia, particularly to the maintenance of soil fertility and to mineral nutrition, two phases of agriculture which are so prominently developed in Russia today. He established at the Agricultural Academy a laboratory for the study of mineral nutrition in 1872. His book "L'Agronomie et la Physiologie Végétale" did much to establish the practical applications of his physiological researches.

The Academy was closed by the government in 1892 under the suspicion of sedition and on account of sympathy with the movements of the agricultural classes which had been a continual source of trouble for the Tsar's government since 1872. The appointment of TIMIRIAZEFF was withdrawn. The following year he occupied himself in the organization of the Congress of Naturalists at Moscow, of which he was elected president. In the years next following, although deprived of his university connections, he continued publication on agronomic subjects and also devoted his energies to the establishment of the Darwinian theory in Russia. During these years he did much to popularize natural science.

He was reestablished in the chair of Anatomy and Plant Physiology at Moscow University in 1901, but resigned in the following year to devote his time to the botanical laboratory. In 1903 he gave the Croonian lecture before the Royal Society of London, summarizing in a clear manner the results of his thirty-five years of research under the title "The Cosmical Functions of the Green Plant." He was honored with the degree of LL.D. by the University of Glasgow in 1901 and received the honorary D. Sc. from the Universities of Geneva, and of Cambridge and Oxford. In 1911 he again resigned from the faculty at Moscow University as a result of the conflict of a number of the university professors with the ministry of public instruction.

From this time on he was unable to undertake exhausting tasks on account of ill health. On the occasion of his seventieth birthday he declined a public celebration in his honor on account of failing health.

TIMIRIAZEFF was a strong advocate of the freedom of thought in the university. This gained for him the following of the more venturesome spirits of the age in which he lived. When this group came into power after the revolution it perpetuated his memory in a manner eminently fitting. The Agricultural Institute in the suburbs of Moscow was named in recognition of his influence in the field of Agriculture.

This academy was founded in 1872 as the first Russian Agricultural Higher School (Petrovskoe-Rasumovskaya Academia) in the grounds of the castle built in 1678 as a residence for the future Tsar Peter I. The college buildings have been repaired and new laboratories for chemistry and

FIG. 1. Library and Administration Building before the park, and to the right, the Klub, a former church at the TIMIRIAZEFF Agricultural Institute, Moscow. Photo by HARVEY.

dairying erected since the revolution. The Academy provides for the instruction of more than three thousand students. The laboratory of plant physiology has been disorganized in the last two years since no appointment has been made to the vacant professorship. The mineral nutrition work of DR. PRIANISHNIKOV occupied five large and well equipped laboratories and had extensive greenhouse space. Interesting work was in progress on root excretion and upon sterile water cultures. There are eight major buildings at the Academy including a good library (see fig. 1). There are extensive plots for plant breeding. The old church of the Tsar, beautifully built in the old Russian style, has been transformed into a social club.

FIG. 2. Statue of TIMIRIAZEFF at TIMIRIAZEFF Circle in Moscow. Photo by HARVEY.

At the TIMIRIAZEFF Circle at Moscow there was erected in 1921 a statue of TIMIRIAZEFF in gray granite, the face an excellent portrait, giving evidence of the nobility of his character. (See fig. 2).—R. B. HARVEY, *Cambridge University, England.*

NOTES

Institute of Chemistry.—The second Institute of Chemistry will be held at Northwestern University, Evanston, Illinois, from July 23 to August 18, 1928. Dr. Harry N. Holmes, of Oberlin College, will occupy one week in the discussion of colloids, and Prof. Whitmore, of Northwestern, will lecture on modern organic chemistry. Other lectures will be given by Dr. B. S. Hopkins, of Illinois, on Inorganic Chemistry; by Dr. A. I. Kendall, of the Northwestern University Medical School, on the Chemistry of Bacteria; and by Dr. Victor K. La Mer, of Columbia University, who will discuss the modern developments in Physical Chemistry.

The industrial chemistry will be of minor interest to plant physiologists. This phase of modern chemistry will be discussed by Dr. W. T. Read, of the Texas Technological College, and Dr. Gerald L. Wendt, of the Battelle Memorial Institute, who will consider particularly industrial research as related to modern industry.

The Chemical Institute held at Pennsylvania State College last year was a great success—not financially, but in what it accomplished. The second Institute will be worth attending by those who desire to keep abreast of the modern trends of fundamental chemical research.

Colloid Symposium.—One of the valuable features of the sixth National Colloid Symposium at the University of Toronto is an exhibition of experimental methods in colloidal work. Such displays play an important part in the rapid development of scientific research, and with the wide interest now shown in colloidal phenomena, this exhibition will no doubt be a very valuable feature of the sixth symposium.

Summer Meeting.—The Purdue Section of the American Society of Plant Physiologists some time ago extended an invitation to the Society to hold a summer meeting at Purdue University during the late summer or early autumn of 1928. The date suggested is September 4–5, and the Executive Committee has voted to accept the invitation. Previous summer meetings have been very successful, and the meeting at Purdue will be of more than usual interest. More details as to the time of meeting will be given in the July number of Plant Physiology.

Dr. Patrick J. O'Gara.—The American Society of Plant Physiologists records with regret the first loss it has sustained by death of a member. Dr. PATRICK J. O'GARA, formerly connected with the investigations in the Rogue River Valley, Oregon, and for many years director of the agricultural and smelter byproducts investigations of the American Smelting and Refining Co., at Salt Lake City, Utah, died on September 17, 1927, at the age of 55 years. His work was mainly in the field of plant pathology and plant physiology, with special reference to the effects of sulphur dioxide and other smelter wastes on agricultural plants. Appropriate resolutions were drawn up at the Nashville meeting, and made a part of the permanent records of the Society.

Stephen Hales Prize.—The committee on the award of the first STEPHEN HALES Prize at the fifth annual meeting in New York, has recently been appointed by the president of the Society. The chairman of the committee is Dr. JAMES BERTRAM OVERTON, of the University of Wisconsin, and the other members are Dr. C. R. BALL, of the U. S. Department of Agriculture, and Dr. A. L. BAKKE, of the Iowa State College. The committee is charged with the selection of a worthy recipient of the prize sometime prior to the New York meeting, and the announcement of the award will probably be made at the annual dinner for all plant physiologists.

The Membership List.—The members of the American Society of Plant Physiologists have received a copy of the membership list published by the Secretary some time ago. The steady growth of the Society is very gratifying. No doubt many of the members know of friends who should be invited to become active members of the Society. The development of the journal is going forward as rapidly as its support makes such a program possible. The library subscriptions have become a splendid source of support, and will no doubt increase in numbers with time. Each member should help to place PLANT PHYSIOLOGY in the library of the institution he serves, so that a permanent and complete file may be available at the institution library.

Purdue Section.—The Purdue Section is an active and vigorous unit of the American Society of Plant Physiologists. The president of the local organization is Prof. C. L. PORTER, who has taken an interest in the development of the Section ever since it was organized. The experience of the group at Purdue shows that these local groups can perform a splendid

service in the institutions where they are active. Other universities would no doubt profit by such sectional activities wherever there are enough members to permit the formation of a section.

Bibliography of Seed Germination.—The Committee for Publication and Registration of the International Seed Testing Association has compiled a "bibliography of germination of seed," which will prove to be unusually valuable to those interested in the physiology of germination and related problems.

The table of contents is published in six languages, English, German, French, Danish, Italian and Dutch. The main headings under which the literature is classified, are as follows: A. Influence of physical factors on germination; B. Influence of chemical factors on germination; C. Influence of living factors (parasites, microbes, injurious insects); D. Influence of state of development; E. Chemistry of germination; F. Technique of germination tests; G. Publications on germination without further indications; and I. Agricultural seeds. The list of authors contains the names of about 1700 investigators and the literature citations occupy 212 pages, including a supplementary list.

Under each main heading, the chronological order of citation is followed. The earliest papers go back to the earlier part of the 19th century, and the latest ones are dated 1928. The list is not without omissions, but is so large as to be extremely helpful to students of germination behavior.

The list is mimeographed, and should not be very expensive if it is planned to place it on sale. The chairman of the Committee is Dr. W. J. Franck, of Wageningen, Holland. The committee has performed a very useful service in preparing this extensive bibliography, and the service will be appreciated by all who have occasion to consult the literature of seed germination.

International Critical Tables.—The third volume of this monumental work has come from the press of the McGraw-Hill Book Co. The volume covers two general fields: the pressure-volume-temperature relations of one-phase systems (density, specific gravity, thermal expansion, and compressibility); and phase equilibria data. The first section occupies 199 pages, and considers, among other things, the pressure-volume-temperature relations of substances which are gases at 0° C. and one atmosphere pressure; nitrogen; thermal expansion of inorganic compounds in the liquid state; of liquid organic compounds; of aqueous solutions of inorganic substances and strong electrolytes; compressibility data for water, alcohols, crystals, pure metals; and density and specific gravity of water.

The second section contains vapor pressure data for a number of the elements, P, S, Se, Te, and the halogens, atmospheric gases, metals, chemical compounds in the crystalline state, ice and water, organic liquids, etc.

There are also tables of partial pressure, vapor pressure lowering, vapor pressure of acids in aqueous solution, and a number of tables devoted to boiling point data with non-volatile solutes. Solubility data are given for gases in liquids and in solutions, for liquids in liquids, and miscibility data with three components. There is also a section on the relative efficiency of drying agents.

The volume contains 444 pages of tables and other information, much of which is occasionally useful to investigators in the biological field. Every institutional library will want a complete set of these volumes.

Indicators.—Although FURMAN'S translation of KOLTHOFF'S work on Indicators has been available for about two years, attention is called to it because of its usefulness in connection with the theory of indicator action. There are eight chapters in the volume, as follows: Neutralization; amphoteric compounds; the color change of indicators; the use of indicators in quantitative neutralizations; the colorimetric determination of hydrogen-ion concentration; practical applications of the colorimetric determination of hydrogen-ion concentration; indicator papers; and theory of indicators. Several useful tables are found at the back of the book, the dissociation constants of water at different temperatures, average degree of dissociation of salts at 18° C., dissociation constants of important acids and bases, and the transition range of indicators.

The book is a valuable one and belongs on the convenient book shelf. It is published by Wiley, 1926, and the cost is $3.50.

Potentiometric Titrations.—This book also is a translation of Dr. I. M. KOLTHOFF'S work by Prof. N. HOWELL FURMAN, of Princeton University. The first six chapters, Part I, treat the fundamental principles of potentiometric work. The chapters bear the following headings: Principles of precipitation and neutralization reactions; electrode potentials, oxidation and reduction; oxidation and reduction reactions and their equilibrium constants; relation between the change of the ion concentrations and electrode potential in combinations of ions; the relation between change in the ratio oxidant: reductant and the electrode potential in oxidation-reduction reactions; and general conditions for the performance of potentiometric titrations.

Part II contains five chapters. The first two of these deal with general matters connected with the technique of potentiometric titrations, and the detection of end points of potentiometric determinations. The last three chapters are devoted to precipitation and complex-formation with metallic or non-metallic electrodes, such as silver, mercury, copper and iodine; neutralizations; and oxidation-reduction reactions.

Sixteen pages of citations, and seven tables, complete the work. It is a companion volume to the one on Indicators, and with the latter forms a splendid two volume work. The price of Potentiometric Titrations is $4.50, and the publishers are Wiley and Sons, New York.

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EFFECTS OF THE ABSENCE OF BORON AND OF SOME OTHER ESSENTIAL ELEMENTS ON THE CELL AND TISSUE STRUCTURE OF THE ROOT TIPS OF *PISUM SATIVUM*

A. L. SOMMER AND HELEN SOROKIN

(WITH FIVE PLATES)

Introduction

While the rôle of the various elements in the growth of the plant has attracted the attention of a large group of workers since the beginning of modern botany, the real function of certain of these elements still remains one of the least understood phases of plant physiology.

Relatively early in the history of the investigation of the growth of plants in culture solution, certain elements were found to be "essential" while others appeared to be non-essential for the normal development of the organism. Obviously, however, it is far easier to demonstrate that an element which is required in relatively large quantities is essential than to prove that elements are not necessary at all. In consequence of the difficulty of establishing the need of plants for certain elements which are required in very small quantities, the list of the "essential" elements was very short, while by implication the list of "non-essential" elements included all the rest.

At the present time the more refined purification of the so-called "chemically pure" salts, and more carefully controlled methods of physiological research, permit a study of the effects of the absence from culture solutions of certain elements with greater exactness than before, with the result that elements once considered unnecessary are being shown to be indispensable for the normal development and functioning of the plant.

The present study forms a part of the work on the essential nature of boron and certain other elements for higher green plants which one of the

writers (7, 8, 9) has been carrying on for the past three years. It represents, however, a cooperative extension of this work into a relatively new field.

The great bulk of the research on the influence of the different essential elements on the development of the plant has employed, as a sole criterion, the mass of tissue produced by plants grown in solutions of varying composition and concentration. This represents only a limited front of attack on the general problem. An ultimate goal should be the determination of the actual rôle of each of the elements in the metabolism of the plant, and the consequent influence of these elements on the morphology of the cell and of the tissues.

While a study of the structure of the cell and the tissues can not furnish final criteria of the rôle of the various elements in the metabolism of the plant, it may represent the first practicable steps toward this end. The work upon which this paper was based was undertaken with this end in view.

Boron seems an unusually favorable element to be employed in a first study of this kind. It was early noted (8) that many dicotyledonous plants when grown in solutions without boron developed short thick roots with enlarged apices. It seemed, therefore, that a study of the pathological changes which take place in these cells and tissues might give some clue as to the function of boron in plant growth.

Both the chemical and cytological phases of such a problem demand refined, delicate and specialized technique. The interpretation of the results requires a knowledge of two fields of literature. At the suggestion of Dr. J. ARTHUR HARRIS, whose interest in and encouragement of this work we have greatly appreciated, we therefore undertook a cooperative investigation of the problem.

Pisum sativum (variety Golden Vine) was chosen as the experimental plant because it was found to show the effects of the absence of boron within a few days and also to produce root tips of a very suitable size for cytological work.

In first experiments plants were grown in a satisfactory culture solution to which boron was or was not added. This solution was the same as that described later as the control solution.

As work on the problem progressed it became evident that a study of the effect of the absence from the culture solutions of some other of the essential elements would be extremely desirable, since such experiments might throw some light on the problem involved. Therefore in a series of cultures most of the elements essential for plant growth were excluded one at a time from the solutions, and the effects of the absence of the various elements in the absence and presence of boron were studied.

Methods

Culture methods

All plants were grown in solutions of highly purified salts. Quart Mason jars were usually employed as containers. In a few instances pyrex breakers were used. The exceptional cases will be discussed later.

This investigation included cultures with and without each of the following elements: boron, magnesium, sulphur, manganese, potassium, nitrogen, iron, phosphorus, and calcium. Each of the last eight groups included cultures with and without boron.

Although zinc was shown by MAZÉ (3) to be essential for maize and by one of us (7, 9) to be essential for several other plants, it was not included in this investigation which deals primarily with the histology and cytology of the root, because of the fact that, in the absence of zinc, the roots appear normal even after the tops are apparently dead. The results caused by the absence of calcium were so striking and so different from anything published so far that they are being reserved for another paper which is ready for publication.

The cultures without potassium were divided into two subgroups, one in which potassium was replaced by sodium and one in which no sodium was added. Pyrex beakers were used as containers in experiments omitting sodium to preclude the possibility of the introduction of this element by solution from soft glass. The purpose of this division was to determine whether any effect of the replacement of potassium by sodium could be observed. In other experiments without potassium (where the effect of sodium was not being studied), Mason jars were employed.

Solutions used were:

Control solution

	Per liter		Gm. per liter
KNO_3	0.80 gm.	Mn (as $MnSO_4$)	0.0015
KH_2PO_4	0.15 gm.	Al (as $Al_2(So_4)_3$)	0.0005
$MgSO_4 \cdot 7H_2O$	0.50 gm.	Cu (as $CuSo_4$)	0.000125
$CaSO_4$ saturated solution	300 cc.	I (as KI)	0.00025
B (as H_3BO_3)	0.0005 gm.	F (as KF)	0.00025
		NaCl	0.0127

Iron (as $FeSO_4$) was added at intervals as required by the plants. Manganese, iron, and boron were omitted from the solution given above in studies concerning the absence of these elements.

Solution without magnesium

	Per liter	
KNO_3	1.20 gm.	B, Mn, Al, Cu, I, F and NaCl as in control solution.
KH_2PO_4	0.15 gm.	
$CaSO_4$ saturated solution	300 cc.	

Solution without sulphur

	Per liter	
KNO_3	1.00 gm.	Traces of the various elements were added as chlorides or nitrates instead of sulphates.
$Ca(NO_3)_2$	0.60 gm.	Fe as citrate.
$MgHPO_4$	0.20 gm.	

Solutions without potassium

I

	Per liter	
$NaNO_3$	1.00 gm.	Traces of various elements as in control excepting that I was added as NaI.
$CaSO_4$ saturated solution	300 cc.	
$MgHPO_4$	0.30 gm.	

II

	Per liter	
$Ca(NO_3)_2 \cdot 4H_2O$	1.2 gm.	Traces as above.
$CaSO_4$ saturated solution	300 cc.	
$MgHPO_4$	0.30 gm.	

Solution without nitrogen

	Per liter	
KH_2PO_4	0.15 gm.	Traces of various elements as in control solution.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	
$CaSO_4$ saturated solution	300 cc.	
K_2SO_4	0.80 gm.	

Solution without phosphorus

	Per liter	
KNO_3	0.80 gm.	Traces of various elements as in control solution.
$MgSO_4 \cdot 7H_2O$	0.50 gm.	
$CaSO_4$ saturated solution	300 cc.	

Purification of salts

KNO_3, $MgSO_4$, KH_2PO_4, $Ca(NO_3)_2$, Na_2SO_4, $MnSO_4$ and H_3BO_3 were repurified by repeated recrystallization from pure distilled water. $CaSO_4$ was prepared by dissolving chemically pure $Ca(NO_3)_2$, filtering and precipitating the $CaSO_4$ with chemically pure H_2SO_4. The $CaSO_4$ was then washed free from acid. $FeSO_4$ was prepared by dissolving iron wire (as used for standardization) in chemically pure H_2SO_4, filtering and crystallizing. $MgHPO_4$ was prepared from purified K_2HPO_4 by precipitating with purified $MgSO_4$. The precipitate was then washed many times with pure distilled water. With the exception of $MnSO_4$ and H_3BO_3 none of the substances used only in very small amounts was repurified. NaCl was used in relatively larger quantities but P. W. R. (Powers-Weightman-

Rosengarten) analyzed salts appeared to be pure enough, so this salt was not purified further. $NaNO_3$ was prepared by evaporating pure NaCl with redistilled HNO_3 until all the chlorine was driven off. The $NaNO_3$ was then crystallized. In the investigations without sulphur it was very difficult to exclude boron when this was desired. $FeSO_4$ seemed to be the only soluble salt easily freed from boron, and the lack of boron was shown in sulphur-free solutions only when a minimum amount of iron as the citrate was added.

No test of the purity of the salts was made other than that shown by the ability or failure of plants to develop in solutions made with them but without the addition of boron. This procedure was followed because we have found that many plants respond to smaller quantities of boron than can be determined chemically and because, according to AUER-WELSBACH (**1**), small amounts of this element are very difficult to detect spectroscopically in the presence of relatively large amounts of other elements.

HISTOLOGICAL AND CYTOLOGICAL METHODS

In obtaining the material for the present investigation, two important precautions were observed. First, environmental factors which may influence the structure and general appearance of the cell components were made as nearly alike as possible and, second, an effort was made to take the tissues for fixation under the most normal conditions during periods of most active nuclear division of the meristematic cells. The material was, therefore, always taken on bright days, in the morning between eight and twelve o'clock. In all cases, with the exception of those mentioned separately, the roots were killed after the plants had been in corresponding culture solutions for from two to three weeks. The majority of the experimental series were repeated during different times of the year.

As a killing reagent, NAWASCHIN's modification of FLEMMING's killing solution was used throughout the investigation. As previous experience of one of the writers indicates, this fixing agent is very reliable and gives splendid results with tissues and cell components. Subsequent treatment was that usually required by and given to cytological preparations. Longitudinal sections of the roots were cut 12 μ and 15 μ thick, and were stained with safranin and aniline blue or with HEIDENHAIN or DELAFIELD haematoxylin.

The photomicrographs were taken with a Zeiss achromatic objective [8 mm. ap. 0.20 (A)] and a projection ocular 2. A combination of Wratten B and E filters was employed. All photomicrographs were taken with exactly the same magnification (about 45 diameters).

Results of histological and cytological studies

Plants grown in culture solutions with and without boron are shown in fig. 5, plate VIII. The three plants to the left were grown with boron for about three weeks; those to the right were grown without boron for the same length of time.

Plants grown in standard culture solutions with and without boron

With boron, fig. 1, plate VII.—The apical portion of roots of *P. sativum* grown in culture solutions in the presence of boron show the typical differentiation of the primary meristem into three distinct regions, plerome, periblem and dermatogen. In the region of contact with the promeristem, the root cap is very well developed and is usually about 20 cells in width. The plerome is about 12 to 14 cells in diameter, the cells being $11\ \mu \times 18\ \mu$, and the nuclei about $10\ \mu \times 10\ \mu$. The nuclei occupy a central position in the cell and each contains a conspicuous nucleolus. Mitotic figures are very numerous in both the plerome and the pleriblem. The dermatogen is usually not very much differentiated from the periblem. The diameter of the root tip in the middle of the region of the primary meristem is approximately 28 to 32 cells. Starting with the beginning of the promeristem, the region of intensive cell division extends for a distance of about 20 to 25 cells to the beginning of the region of elongation. The region of maturation usually does not begin at a distance of less than from 60 to 70 cells from the tip.

Without boron, figs. 2–4, plate VII.—The apical portions of roots grown in culture solutions containing the elements usually considered essential, but without boron, show remarkable changes from those described above. The region which corresponds to the primary meristem of the normal root can hardly be called meristem. Progressive changes (in the sense in which this term is used by students of pathological plant anatomy) take place, which entirely modify the appearance as well as the structure of this region. Since the term meristem cannot be properly applied to the region under discussion, it would be logical to avoid the terms plerome and periblem in indicating that portion of the root in which pathological changes take place. But because the terminology of pathological plant anatomy lacks uniformity in general and is entirely inadequate for this special case, we will refer to the regions found in the apical part of roots grown without boron as plerome, periblem, and dermatogen, always keeping in mind the fact that these names are used here, not for regions of primary meristem, but for those homologous with the primary meristem in the normal root.

In the absence of boron the root cap is considerably deformed or even entirely lacking. In the region of the plerome and periblem progressive

changes take place which result in an enormous enlargement of the whole apical portion of the root. Distinct hyperplasia is developed in the plerome, while in the periblem hypertrophy usually obtains. In the central portion of the apical part of the root the plerome region usually extends from about 30 to about 32 cells in diameter, which is about twice as many cells as are present in the corresponding region of roots grown with boron. In the early history of the cultures the cells divide by mitosis but soon lose their ability to divide further by typical mitosis. In the absence of boron the regularity in the arrangement of the cells is lost. The mechanism regulating the divisions of the cells is apparently disturbed, with the result that the cells appear in irregular uncoordinated rows or even without the formation of definite rows. The cells are extremely irregular in shape and size. They range from $10\,\mu \times 15\,\mu$ to $11\,\mu \times 18\,\mu$, and the nuclei are about $6\,\mu \times 7\,\mu$.

The rows of cells in the periblem regions are more regular than in the plerome and correspond in number to those of the normal root. The cells, however, are much larger than normal, being $50\,\mu \times 50\,\mu$ with nuclei $7.5\,\mu \times 8\,\mu$. It is interesting to note that although these cells are much larger than the cells of the normal root, their nuclei are smaller. Returning to the plerome we note that besides the lack of regularity of arrangement and form of the cells, another interesting abnormality appears. The cells of this region undergo premature development which is manifest in the appearance of isolated elements of xylem as far down as the level of the twentieth cell from the apex. These first occur at irregular intervals and show unequally thickened walls which do not take the stain typical for lignified cell walls. The elements occurring slightly farther up stain red with safranin but are extremely irregular in appearance. Incompletely scalariform or scalariform-reticulate xylem elements usually predominate. Besides the abnormal occurrence of these xylem units, the premature development of the tissues is further manifest in the formation of isolated meristematic regions of branch root primordia in different parts of the apical portion of the root. These regions are sometimes formed at a distance of no more than 10 to 13 cells from the apex. However, they very rarely continue their development for any length of time, but are apparently suppressed shortly after they have been formed. This is illustrated in fig. 4, plate VII. The photograph presents a tangential section in which a series of new meristematic regions is shown, most of which were suppressed soon after they were formed. Only the fifth of these incipient lateral roots developed far enough to grow through the periblem. A careful examination of the material shows that typical mitoses are usually absent. In these newly formed meristematic regions, slightly abnormal mitoses are observed.

Another important characteristic of the tissue found in the plerome region is the formation of thick strands which apparently represent the collapsed and thickened walls of cells. These are shown clearly in fig. 3, plate VII. Farther away from the apical portion, a region occurs in which the lignified elements become more prominent and are even connected for more or less regular distances. These, however, may be interrupted by portions in which the connection between the individual elements is lacking. Pitted vessels begin to be more prominent in this region.

Recovery from injury due to the lack of boron

Root tips in the process of recovery from injury due to the lack of boron present a very interesting picture. If boron be added to solutions which have produced plants showing typical symptoms of the lack of boron, but whose tissues are not too badly disintegrated, evidences of recovery appear very quickly.

In a series of experiments boron was added to solutions in which the plants showed an extreme degree of injury due to the lack of this element, and root tips of these cultures were fixed after 6, 24, and 48 hours. A definite zone of truly meristematic cells seems to appear as early as 6 hours after the addition of boron. A newly formed meristematic region is apparent in the tissues fixed at the end of 24 hours. After 48 hours a new long slender growth may be seen at the end of the enlarged apex. This newly formed root apex sometimes grows more than a centimeter in 48 hours, and acquires the diameter and general appearance of the normal root.

Plants grown in solutions without magnesium

With boron, fig. 6, plate VIII.—The root apices of plants grown in solutions lacking only magnesium do not show any noticeable deviation from the normal. Mitotic figures are numerous and regular.

Without boron, fig. 7, plate VIII.—Rather distinct peculiarities were observed when both boron and magnesium were absent which were not seen when boron alone was lacking. We cannot say that these were real differences, and not variations of the effects of the absence of boron alone, since sections of only three root tips were available for study. It is, however, desirable to describe these differences which are as follows: the plerome and periblem regions are less definitely marked off in the most apical portion of the root than those of cultures grown without boron alone. Hypertrophy sometimes occurs in the plerome as well as in the periblem. There are also some other deviations from the typical conditions for the absence of boron. The isolated regions of branch root primordia are not so prominent. The cells of the periblem region appear less hypertrophied than

those of the corresponding region of roots of plants grown without boron only, their size being 25 μ x 25 μ instead of 50 μ x 50 μ. The size of the nuclei is, however, the same in both cases.

PLANTS GROWN IN SOLUTIONS WITHOUT SULPHUR

WITH BORON, FIG. 8, PLATE VIII.—The roots appear more or less normally developed with the exception of the occurrence of rather large intercellular spaces in the periblem region, where whole rows may be missing. A similar phenomenon sometimes occurs normally in certain species of plants but we have not observed it in roots of *P. sativum*. Sometimes single cells are lacking, making the rows discontinuous. This apparently does not occur in the plerome region where the cells appear very normal, and where mitotic figures occur. The size of the cells in the plerome corresponds to those for plants grown in the control solutions.

WITHOUT BORON.—In our first experiments, where an attempt was made to exclude both boron and sulphur we failed to produce the typical effects due to the lack of boron. Contamination of the solution by boron was suspected, since $FeSO_4$ is the only soluble iron salt easily purified and this could not be used because it would add sulphur which we also wished to exclude.

In earlier experiments in which there was probably some contamination by boron, the roots did not differ much from those grown in solutions to which boron had been added, but the intercellular spaces observed in the periblem region when only sulphur was lacking were even more conspicuous. This is well shown in a tangential section of a root, fig. 9, plate VIII. In a later experiment, in which only a very limited supply of iron (as ferric tartrate) was used, and the contamination by boron thus greatly reduced, an approach to the typical effects due to the absence of boron is easily observed.

PLANTS GROWN IN SOLUTIONS WITHOUT MANGANESE

WITH BORON, FIG. 11, PLATE IX.—The size of the root and the regions of meristem is normal. Mitotic figures are numerous and normal. In the region of elongation the cell walls are, however, abnormal, being irregularly thickened in certain parts and loosely coherent in others. A considerable thickening of the transverse cell wall is particularly noticeable.

WITHOUT BORON, FIG. 10, PLATE IX.—In contrast with other cultures grown without boron, roots grown without this element and without manganese have well developed root caps in all cases which were studied. In the meristematic region of the primary root, the nuclei divide by mitosis, but some irregularities in the process of division are observed. The metaphases appear very much deformed. An orientation of the spindle nor-

mally parallel to the longitudinal axis is seldom observed. Figures in which anaphases and telophases can be recognized are almost entirely lacking in the root apices, but reduced anaphases and telophases are present in the secondary roots. These stages seem, however, to occur only during the early period of the development of the laterals. There is some evidence that, after the plants have been growing for a considerable length of time in the absence of manganese and boron, the nuclei of the cells of the principal root apex begin to divide by pseudoamitosis, or fail to divide at all. This may, however, be a general phenomenon common to the absence of several of the essential elements.

The pathological changes of the tissues consisted in considerably developed hyperplasia of the cells of the plerome, and hypertrophy of the cells of the periblem. The premature development of the cells is very pronounced, the isolated and incompletely developed xylem elements appearing early. The formation of secondary roots occurs very close to the meristematic region. The cell walls appear to be extremely thick in certain cells, which is clearly shown in the periblem of the upper part of the photomicrograph, fig. 10, plate IX.

Plants grown in solutions without potassium

As mentioned in the paragraph describing the solutions used in this investigation, groups of the plants in the work with potassium were employed in order to determine whether there was any visible effect of the replacement of potassium by sodium. The cytological and histological pictures were the same in both cases, so what is said for one solution holds for the other also. Pyrex containers were used for these cultures to prevent the introduction of sodium from the glass. Since pyrex is a borosilicate, enough boron was supplied to the boron free solutions from the containers to prevent the symptoms of the lack of boron from appearing for some time. Within a month, however, plants growing in solutions in pyrex containers to which no boron had been added began to show the characteristics produced by the absence of boron. In experiments in which ordinary Mason jars were used, the effects of the absence of boron appeared in the early stages of growth.

With boron, fig. 12, plate IX.—The roots exhibited normal differentiation of the meristematic tissue into plerome, periblem, and dermatogen. The root cap was very well developed and typical mitoses occurred frequently in the meristem. It differed from the control in that there was a somewhat loose arrangement of the cells in certain parts of the roots and the occasional dropping out of entire rows. These results differ from those obtained by Reed (5) for *Spirogyra* which was cultured in the absence of potassium and in which mitotic divisions were not observed. His plants

were kept in potassium free solutions for thirty-five days, while ours were without potassium other than what was stored in the seed for less than three weeks. Whether mitotic division in his *Spirogyra* plants ceased because other processes had been too adversely affected, whereas ours had not yet reached this stage, or whether these very different types of plants respond differently to the absence of potassium is a matter for further investigation.

The rôle of potassium in the metabolism of the cell has been studied by MACALLUM (**2**), and WEEVERS (**11**). See MOLISCH (**4**), pp. 63–64. These authors succeeded in demonstrating, by microtechnique, the presence of this element in the cytoplasm of cells of all plants investigated except in those of the *Cyanophyceae*. Their reaction for potassium in the nucleus was negative, and from this fact they concluded that potassium as a rule is absent from the nuclei. Their test, however, would not show potassium in organic combination, and although we do not know that such compounds exist in the plant, we still have to consider this possibility.

Laws of physical chemical equilibrium make it appear impossible that the potassium ion would be entirely absent from the nucleus when it is present in the surrounding cytoplasm, and furthermore, a colloidal substance with such elasticity as SEIFRIZ (**6**) has shown the nucleus of *Cryptobranchus* to have, would very probably prevent the precipitation of the potassium compound in particles large enough to be seen with an ordinary microscope.

In addition to the results in the papers mentioned above, WEEVERS (**11**) found that potassium was especially abundant in the cell vacuole and absent in the chromatophores. Potassium was conspicuous in the growing points and storage organs of the phanerogams. The reaction showed plainly in the sieve tubes, but poorly in the other vascular elements. A considerable amount of potassium was demonstrated in the cambium and medullary rays. MOLISCH (**4**), p. 64, comes to the general conclusion that, although it is difficult as yet to decide what the exact rôle of potassium in cell metabolism may be, there are many indications that this element plays a part in the building up of the plasma of the growing points, and that it is important in the development of turgor in the cells.

WITHOUT BORON, FIGS. 13, PLATE IX, and 14 and 15, PLATE X.—The typical pathological changes due to the lack of boron in the series of experiments from which both boron and potassium were excluded were not obtained at once in the first experiment. The difficulty may be attributed to the fact that in attempting to grow plants in pyrex containers sufficient boron may be introduced from the glass for normal development during the early stages of growth. Another difficulty was that the salts used in this experiment were not quite free from boron. Finally both difficulties were over-

come and a typical demonstration of the abnormalities due to the absence of boron was obtained.

A longitudinal section of the apical portion of a root is represented in fig. 13, plate IX. The meristematic region is abnormal but not entirely abortive. It is represented in the photomicrograph as a very dark area. The nuclei show division by mitosis, in which all of the phases were observed, but there is a deviation from normal mitosis in the somewhat irregular appearance of the chromosomes. Hypertrophy and hyperplasia of the periblem and plerome respectively were distinct, but not as extensive as in the cultures lacking boron only. The region showing hyperplasia was only 22 cells in diameter as compared with 30 to 32 cells in the corresponding region of root tips grown in the absence of boron only. The premature development of the root regions was not as distinct as in the cultures lacking boron alone. The lignified elements of the xylem, as represented in fig. 13, are at a region less than two thirds of the distance from the apex as compared with one sixth of the distance where boron alone was excluded. The abnormal formation of branch root primordia was not observed. Root apices from plants grown in solutions from which both boron and potassium were excluded appeared less deformed than those of plants in which only boron was absent. Here a slight contamination of the culture medium may explain the differences.

A longitudinal section of the apical portion of a root grown in a pyrex container for two weeks is shown in fig. 14, plate X. There is practically no difference between this root and those grown in the control solutions. A root from the same culture which was grown for a month and a half is represented in fig. 15, plate X. Although it is not a typical picture of a root grown in the complete absence of boron there is considerable resemblance. Pyrex glass evidently yielded sufficient boron for normal development in the early stages of growth but not enough to supply the plants for continued development.

Plants grown in solutions without nitrogen

With boron, figs. 16 and 17, plate X.—The typical characteristics of roots of pea plants grown in solutions without nitrogen but in the presence of all other essential elements is that they are comparatively long and of small diameter. Sections of the same root are shown in figs. 16 and 17. The section represented by fig. 16 is not in immediate continuation of that given as fig. 17, there having been omitted from between these two sections another part as long as that in fig. 17 and similar in structure to the upper part in fig. 17.

The root cap is very poorly developed. The meristematic region is present and the mitotic figures are distinct, but the cells appear disarranged,

especially in the periblem region, where large intercellular spaces may be observed. The general orientation of the rows is apparently very much affected by the absence of nitrogen. The region of mutation appears to be present at a normal distance from the root apex. The elements of the xylem appear to be connected in the regular manner. The secondary roots, when formed, are deflected downward to an unusual degree and destroy the cortex not only where they protrude but also in the region immediately surrounding this part. The cells of the plerome region are 10 μ x 20 μ; the nuclei are 10 μ x 10 μ. The nuclei are regular and spherical and appear normal. In the periblem region, the cells are about 30 μ x 15 μ, very irregular and in sections often without visible connection with the neighboring cells. The nuclei, however, are not diminished in size and are similar to the nuclei of the plerome region.

WITHOUT BORON, FIGS. 18 and 19, PLATE X.—The differentiation of the regions which are more or less distinct in case of plants grown without nitrogen and with boron, is less obvious in the roots of plants grown without boron. As a rule the progressive tissue changes which occur in these roots generally take the form of hyperplasia, which in this case occurs in the region corresponding to the plerome as well as the periblem. The cells are very small and their number correspondingly great. In certain parts of the root apices the diameter is more than 50 cells in width as compared with 28 to 32 cells in the normal root and 46 to 50 cells where boron only is absent.

The cells which undergo hyperplasia are very small and extremely irregular in size, ranging from 5 μ x 7 μ to 10 μ x 10 μ. Many of the cells could not be measured because their outlines were so indefinite. The nuclei are very small in the region of hyperplasia, being about 4 μ x 5 μ. In the parts where a slight differentiation into regions of primary meristem have taken place, the cells of the periblem are 15 μ x 22 μ and the nuclei are 6.25 μ x 6.25 μ.

In contrast to the roots of plants grown without nitrogen but with boron, the cells of the periblem region, when such a region becomes differentiated, occur in more or less regular rows. The tissue of the plerome, however, shows extreme irregularity in the arrangement of the cells. The typical rows could be distinguished very rarely and the cells varied greatly in size and shape. In a few cases, however, as represented in fig. 18, plate X, the hyperplasia was not developed to so great an extent, and hypertrophy was more pronounced.

PLANTS GROWN IN SOLUTIONS WITHOUT IRON

WITH BORON, FIG. 21, PLATE XI.—The root tip did not differ from the control; mitotic figures were numerous and normal. The size of the cells and of the nuclei was the same as that of the controls.

WITHOUT BORON, FIG. 20, PLATE XI.—Root tips of plants grown in solutions without iron and without boron showed the same characteristics as those of plants grown in solutions from which boron only was absent. No mitoses were observed in the apical part of the root, but well developed mitoses were present in the meristematic region of the secondary roots, as shown in fig. 20, plate XI. A few well developed metaphases and other stages of nuclear division are present even in the central cylinder of the primary roots.

PLANTS GROWN IN SOLUTIONS WITHOUT PHOSPHORUS

WITH BORON, FIG. 22, PLATE XI.—The external form of the roots of plants, grown for from two to three weeks in solutions without phosphorus but with boron, does not appear very much modified in comparison with normal roots. A study of the tissues showed that the arrangement of the cells was regular. Within the cells, however, the abnormalities were pronounced. The same fixing fluid which proved to be so excellent for all other cases studied apparently gave sections exhibiting plasmolysis. We have as yet no means of telling whether this condition was caused by the fixing agent or whether it occurred before the tissue was killed.

The nuclei are the structures most affected by the absence of phosphorus. They lose their typical more or less spherical shape and assume an extremely irregular outline. Amoeboid, elongated, spindle shaped and constricted nuclei are very often found in the meristematic region. Some of the nuclei are much smaller than those of normal cells. Instead of occupying a position in the center of the cell, as is characteristic of nuclei in meristematic tissue, they often appear flattened against the transverse cell wall. Normal mitotic figures were not observed. The nuclei sometimes appeared more normal in the region of elongation of the root.

Somewhat similar effects on the cells of lower plants have been described by REED (5). He found that not only were mitoses absent in plants of *Spirogyra* which were growing for three weeks in phosphorus free solutions, but that the cells grown for a certain length of time in such solutions were difficult to stimulate to divide when they were placed in solutions containing phosphorus. According to REED, cells transferred to phosphorus free solutions first lose the soluble phosphorus compounds and later show injury in the living parts of the cells.

In our experiments changes in the root tips of the peas described above took place in plants which were kept in phosphorus free solutions for from two to three weeks. Plants kept under similar conditions for a shorter time showed mitotic divisions of the nuclei which were normal or only slightly abnormal. The fact that peas can be grown in phosphorus free solutions for as long as four or more weeks is difficult to explain in

view of the fact that plants with comparatively minor injuries to the cells caused by the absence of boron die in so short a time.

It must be remembered, however, that the influence of the absence or presence of a given element can not be judged wholly by its morphological effects on the cell and tissue structure. The effect of boron on the cells and tissue structure may not be fully represented by abnormalities of structure. This influence may be merely secondary, and its real function concerned directly or indirectly with some very vital and more continuous process, such as, for example, respiration.

The whole problem of the effects of the absence of phosphorus, as well as of the absence of many of the other essential elements, requires much additional investigation.

WITHOUT BORON, FIG. 23, PLATE XI.—Pathological changes in the tissues of plants grown in the absence of both phosphorus and boron resemble those described for plants lacking boron only. The cells of the central cylinder seem to be most injured. Their protoplasts disintegrate. The process of disintegration ultimately results in the complete dissolution of the nucleus and cytoplasm. The disintegration of certain individual cells results in an irregularity of the arrangement of the cells of the central part of the root. The nuclei which retain a nucleolus, even though it may be very inconspicuous, remain alive. These nuclei, however, differ from those typical of meristematic tissues in that they resemble a coarse precipitate instead of the finely granular structure of the typical resting nucleus.

Discussion

In conducting the present investigation our primary object was to determine the changes in the cells and tissues of the root tips of *P. sativum* induced by the absence of boron from the culture solutions. To make these tests more critical, certain other elements recognized as essential, namely magnesium, sulphur, manganese, potassium, nitrogen, iron, phosphorus, and calcium, were omitted one at a time from the media. These studies, in which elements other than boron were excluded, were carried out only to determine whether or not their absence would influence the typical effects induced by the absence of boron. They are, therefore, very incomplete in so far as the specific effect of their absence is concerned. Each of these elements deserves an extensive, independent investigation from the cytological and histological view point, which, because of the purpose of this problem, was not included in this paper. We feel, however, that the results obtained here incidental to the study of the absence of boron, are of sufficient importance to warrant their being recorded as a basis for future special investigations of the structural changes occurring in the absence of these several elements.

Studies of the effects of the absence of boron in the presence and absence of certain of the other essential elements have shown the structural modifications to be roughly similar for all the elements with the exception of calcium. The absence of calcium caused such marked and rapid changes of the tissues, and its effect anticipated that of the absence of boron, so that if the absence of boron caused any abnormality it could not be observed when both elements were wanting. The remarkable effects of the absence of calcium will be treated separately in a subsequent paper.

Meristematic tissues are the first to show the effects of the absence of boron. On the exclusion of boron from the medium, the factors regulating the process of cell division and the normal orientation of the cells in the tissues no longer function normally. In a relatively short time after the plants are transferred to boron free solutions the cells cease dividing and a general arrest of growth follows. Thus the absence of boron influences not merely the structure of the cell but the organization of the tissue. Although more or less regular mitotic divisions are present in the early stages of root development of plants grown without boron, some modifications of this process occur very soon and result in pathological tissue changes. Hyperplasia becomes very pronounced in the region which corresponds to the plerome of the normal root while a distinct hypertrophy is observed in the periblem. The suppression of mitotic divisons in the absence of boron is not typical for the cells of the whole root. Secondary meristematic regions may be formed in the more apical part of the root. These are, however, usually suppressed during an early stage of development, and true secondary roots seldom develop. Normal mitoses occur at the beginning of these new meristems but soon disappear.

The changes in the anatomical structure of the roots of *Vicia faba* induced by the absence of boron were studied by Miss WARINGTON (**10**). She found that the cells of the cambium were affected first. These cells were shown to lose their regular outline, and peculiar dark streaks were found between them. Their development was also irregular, a few large cells often occurring in place of a number of small ones. This was particularly noticeable in the region of the cambium, and according to Miss WARINGTON's interpretation, was partly due to the breaking down of some individual cells rather than to any enlargement of existing cells. She also noticed the suppressed development of branch root initials, which, owing to the injury sustained from the lack of boron, failed to develop normally. She interpreted the thickened appearance of the roots as due to the irregular development of the pericycle and the formation, but suppressed development, of the root initials above mentioned.

We cannot fully agree with Miss WARINGTON's explanation of the cause of the thickened appearance of the root. The principal cause, ac-

cording to our investigation is the hypertrophy of the periblem and hyperplasia of the plerome regions. The formation of the suppressed root initials may, of course, add some additional thickness, which is usually seen as spherical enlarged areas in the stunted root apex.

The changes in root tips of *P. sativum* induced by the complete or, perhaps better, the nearly complete absence of boron from the culture solution, take place so rapidly that our study is chiefly one of final results. This makes interpretation difficult. It is obvious that a factor or factors regulating growth have been disturbed, but it is impossible, with only this final morphological picture to decide whether this is a direct or an indirect effect. Studies of root tips taken at relatively short intervals after the plants have been transferred to boron free solutions may help in solving the problem by providing a more or less complete series of the intermediate stages.

Another line of attack is suggested by the fact that certain plants, namely the grasses (**8**), (**9**) respond quite differently to the absence of boron from the nutrient media. We are inclined to believe that such differences in response cannot be due only to the amount of boron stored in the seed, but that they are probably concerned with some marked difference in the metabolism of the different plants. We hope that a more extensive comparative study of different forms will give a clew as to what part boron plays in the development of the organism.

Summary

1. Plants of *Pisum sativum* grown in solutions in the absence of boron exhibit short, thick, and stunted roots.
2. Plants grown in culture solutions in the absence of boron and of certain of the other essential elements showed pathological changes roughly similar to those found in the absence of boron only.
3. The enlargement of the root apices is due to the hyperplasia of the plerome and hypertrophy of the periblem regions.
4. The meristematic region of root tips grown without boron becomes abnormal. The cells cease dividing normally and existing cells undergo premature development or pathological changes. Isolated xylem elements appear in regions occupied by the meristem in normal roots, or in the region of elongation.
5. The primordia of the secondary roots begin to be formed abnormally close to the root tip. Usually, however, they are soon suppressed and secondary roots seldom develop.
6. In a general way, we may state that the absence of boron causes a disturbance in the regulation of growth and development.

7. The profound structural changes here observed make it quite clear that physiological investigations of the various elements must be accompanied by morphological, histological and cytological studies to attain their fullest significance.

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EXPLANATION OF PLATES

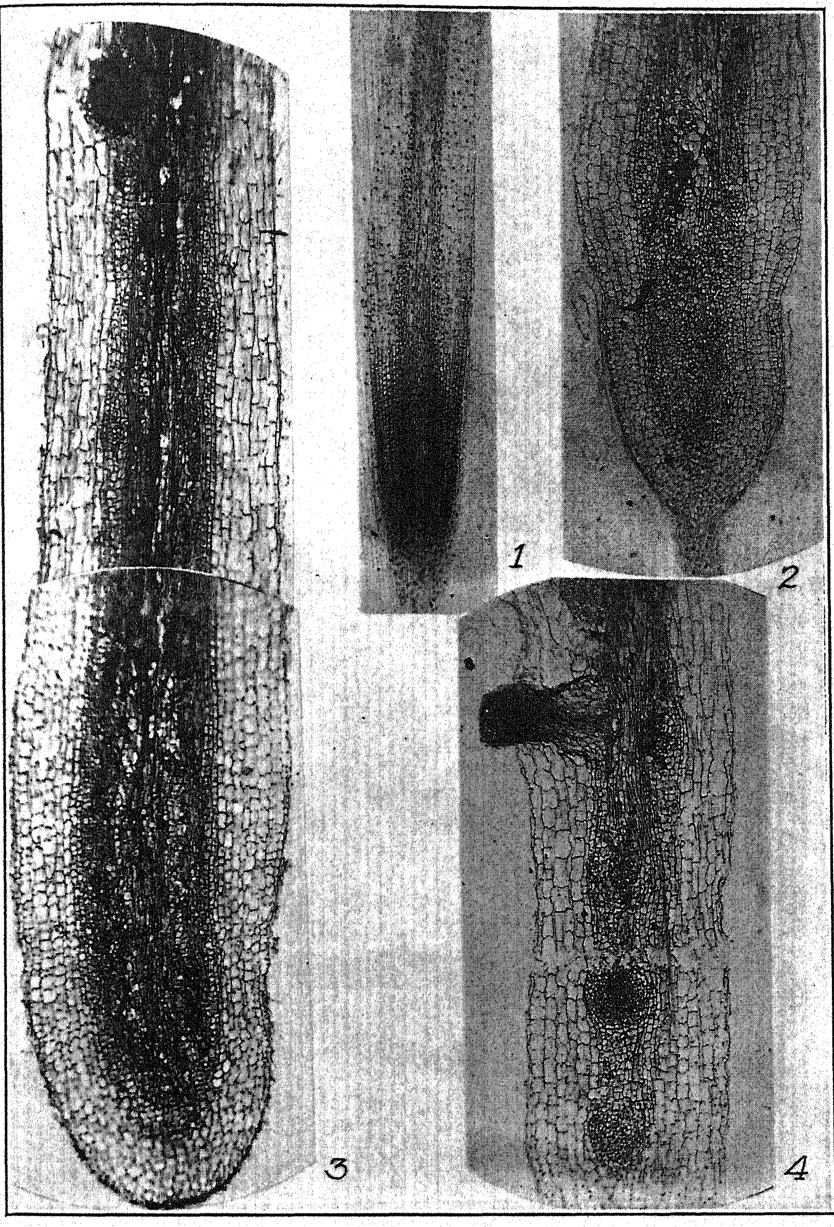
All photomicrographs were taken with a Zeiss achromatic objective [8 mm. ap. 0.20 (A)] and a projection ocular 2. A combination of Wratten B and E filters was employed. All photomicrographs were taken at the same magnification. The experimental plant was *Pisum sativum* (variety Golden Vine). All figures excepting 5, plate VIII, represent longitudinal sections of root apices.

FIG. 1. Grown in control solution; all elements present.

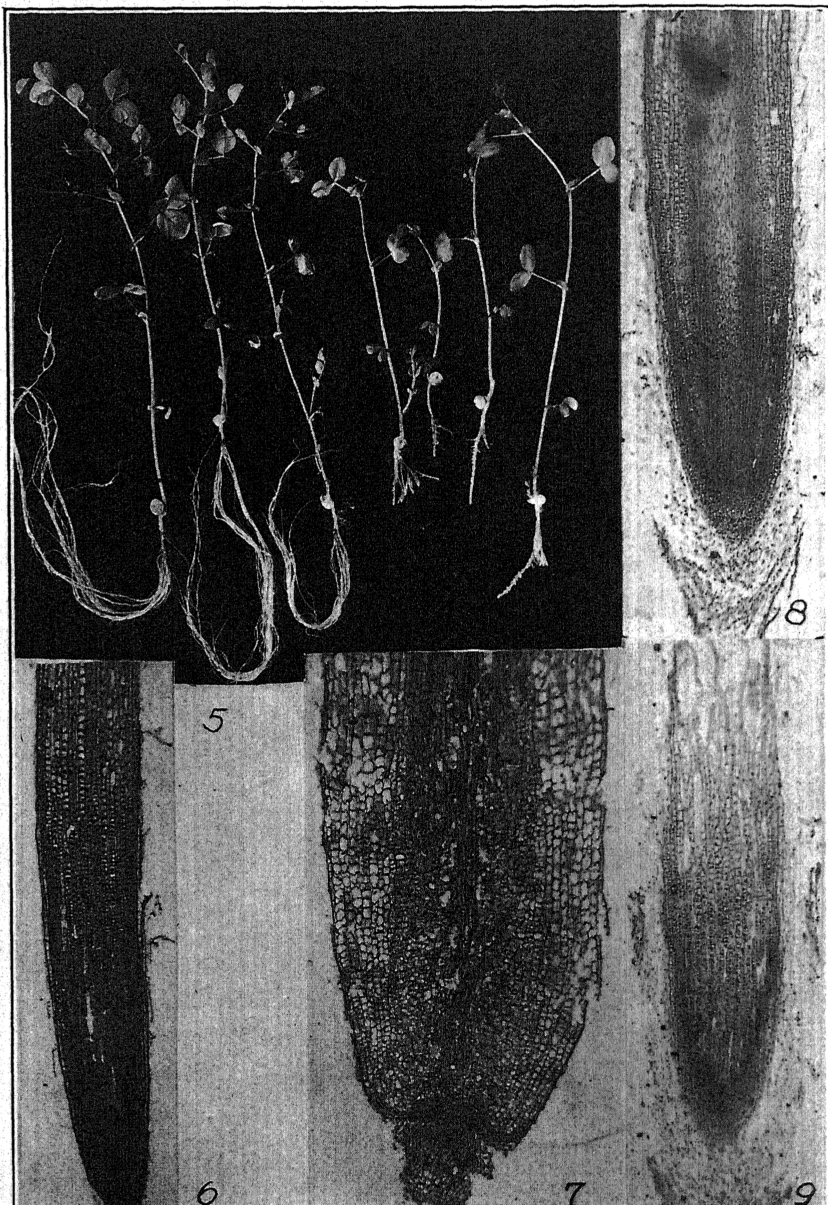
FIG. 2. Grown in the absence of boron.

FIG. 3. As in figure 2. Shows hyperplasia of plerome and hypertrophy of periblem.

FIG. 4. Tangential section of a root grown in the absence of boron. Secondary root primordia are formed very close to the root tip.



SOMMER AND SOROKIN—ABSENCE OF BORON



SOMMER AND SOROKIN—ABSENCE OF BORON

FIG. 5. The four plants to the right were grown in the absence of boron for three weeks. They have very short stunted roots, secondary roots made very little growth. The three plants (controls) to the left were grown in the same kind of solution with the exception that boron had been added.

FIG. 6. Grown in the absence of magnesium and in the presence of boron.

FIG. 7. Grown in the absence of both magnesium and boron. The pathological changes in the tissues are typical for the absence of boron.

FIG. 8. Without sulphur. A slight irregularity may be observed in the periblem. The rows of cells are loosely connected.

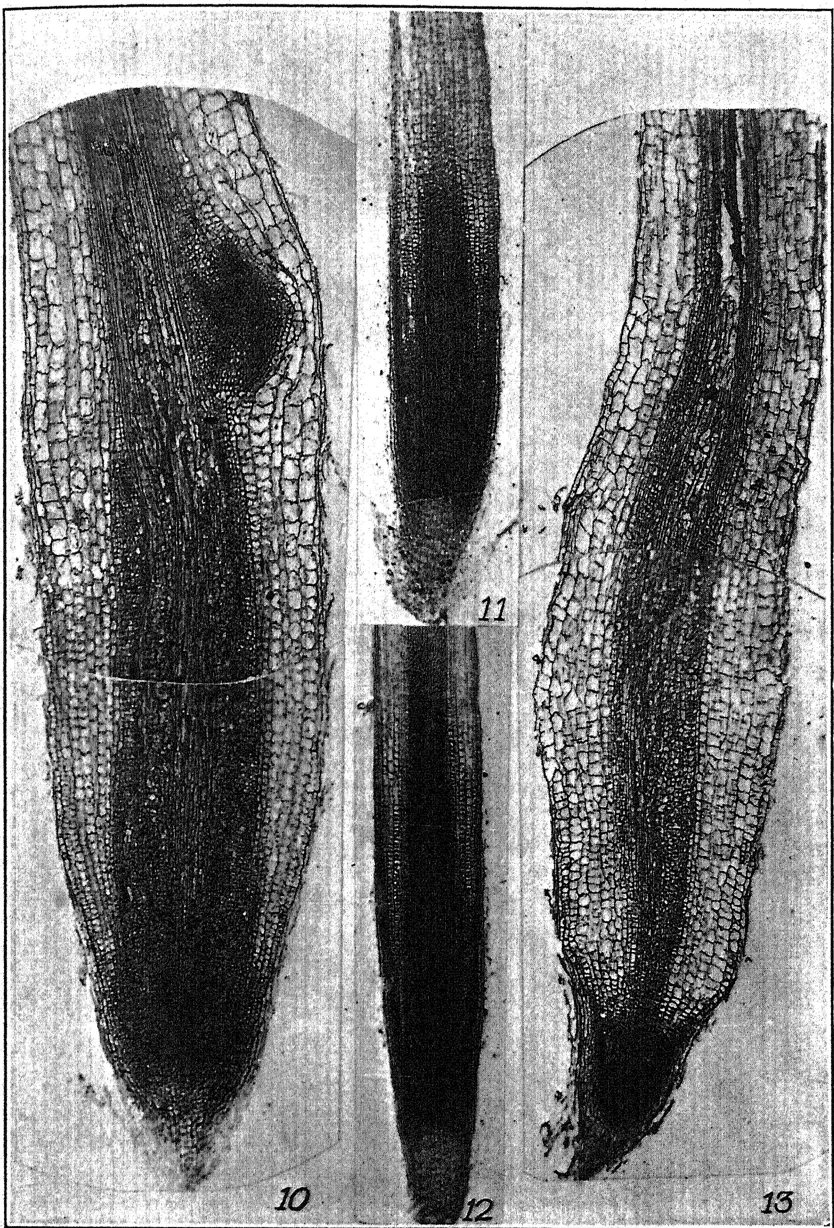
FIG. 9. Without sulphur and with no boron excepting a small amount occurring as an impurity in the salts. The rows of cells are very loosely connected. (A tangential section).

FIG. 10. Without manganese and without boron. Hypertrophy of the periblem and hyperplasia of the plerome are well developed. A branch root primordium may be seen in the upper part of the figure. Irregularly thickened cell walls are present in the periblem.

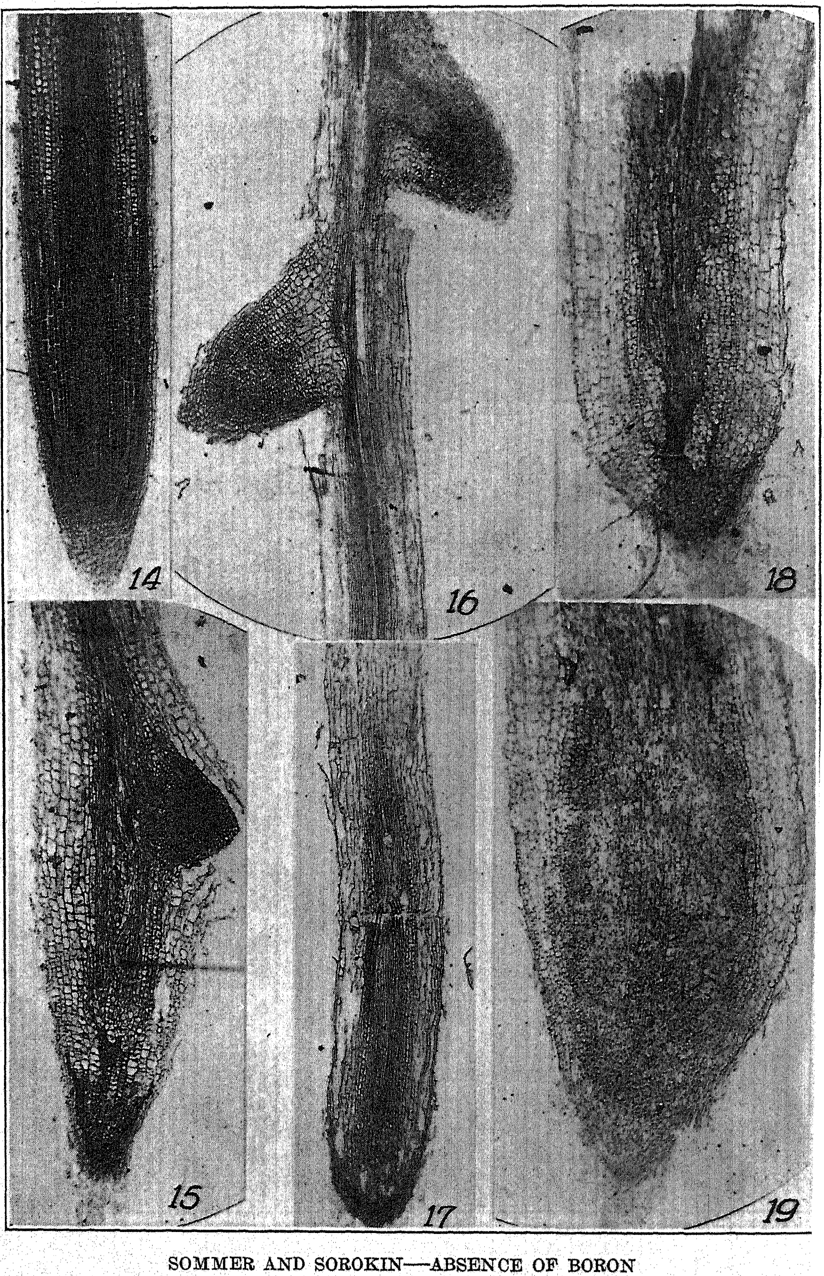
FIG. 11. Without manganese, with boron.

FIG. 12. Without potassium, with boron.

FIG. 13. Without potassium and without boron. Typical effects of the absence of boron are present.



SOMMER AND SOROKIN—ABSENCE OF BORON



SOMMER AND SOROKIN—ABSENCE OF BORON

FIG. 14. Without potassium and without boron. Grown for two weeks in pyrex containers. Pyrex yielded enough boron to supply the plant during the early stages of growth.

FIG. 15. As in fig. 14 but grown in pyrex for six weeks. The root shows some abnormality, but the typical effect of the absence of boron was not yet obtained.

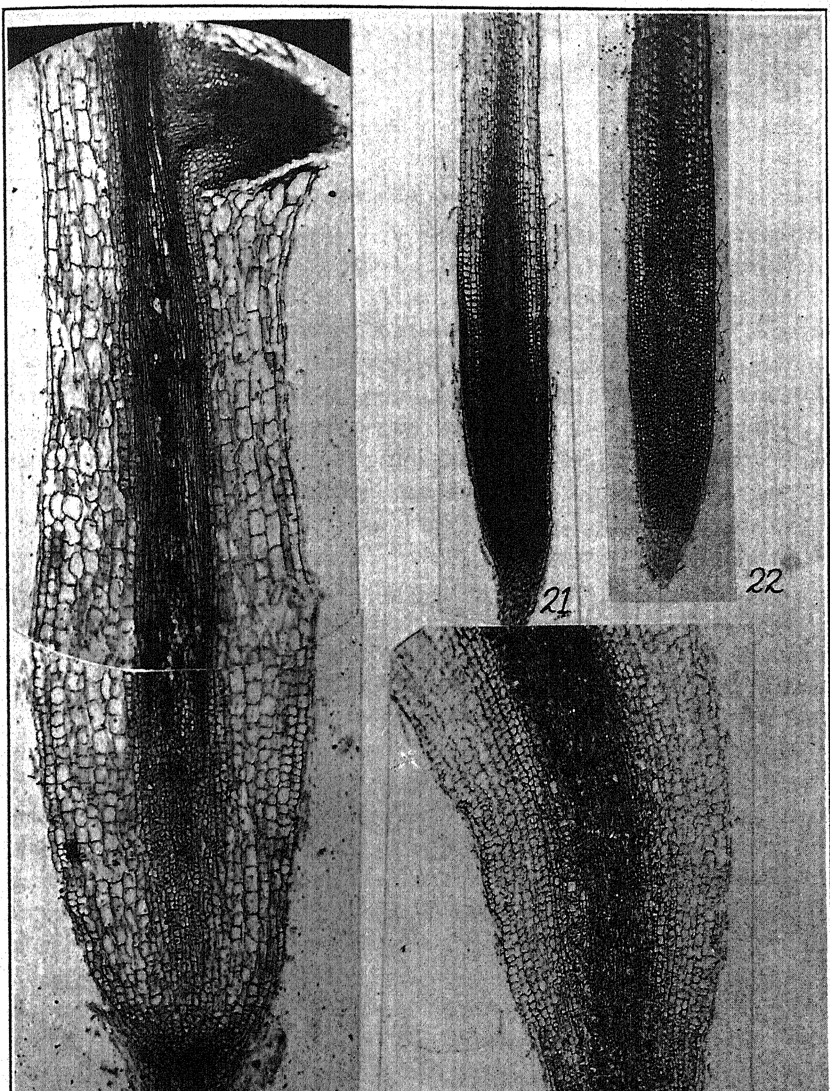
FIG. 16. Without nitrogen, with boron.

FIG. 17. Apical portion of root shown in fig. 16.

FIG. 18. Without nitrogen and without boron.

FIG. 19. As in fig. 18. Hyperplasia of the plerome is very well developed.

FIG. 20. Without iron and without boron. Typical effects of the absence of boron.
FIG. 21. Without iron, with boron.
FIG. 22. Without phosphorus, with boron.
FIG. 23. Without phosphorus and without boron.



SOMMER AND SOROKIN—ABSENCE OF BORON

CALCIUM, POTASSIUM, AND IRON BALANCE IN CERTAIN CROP PLANTS IN RELATION TO THEIR METABOLISM

WALTER F. LOEHWING

Introduction

Due to the widespread use of lime and potash in agricultural fertilizer practice, reports of instances in which these elements have proven injurious are continually increasing. Survey of the voluminous literature on responses of crop plants to mineral fertilizers reveals that injury following lime or potash amendments occurs most commonly when there is extreme lack of balance between these and other nutrient elements in the soil. Though lime and potash injury has been observed most frequently on mineral soils, highly organic soils often exhibit it even though acid in reaction or low in potash. Attempts to increase the fertility of poorly productive organic soils of the muck type in the thickly populated regions of the United States have not in general proven very successful on account of the peculiar physical and chemical characteristics of these soils.

Mucks are somewhat similar to peat soils in origin but their vegetable detritus has undergone such pronounced disintegration that the resulting particles, in contrast to the more bulky peat soils, betray surface phenomena to a remarkable degree. Conspicuous among these are adsorption and capillary effects which often become critical in determining the availability of nutrients and water. These unproductive mucks may superficially resemble productive loams by being dark in color and pulverulent in texture but they differ from them by being extremely light in weight, low in mineral content, high in combustible matter, high in water retentivity, and poor in thermal conductivity. Many of the singular responses of muck soils to fertilizers can undoubtedly be ascribed to their unique physical characteristics.

Earlier reports of fertilizer tests on organic soils showing injurious effects of lime and potash to crops (**7**, **38**, **39**) have not always included complete plant analyses and hence the exact physiological cause of the injury observed has not been understood. Some of these earlier tests (**11**, **19**) covered a period of years in order that the effects of climatic fluctuations from year to year and the errors of immediate response observations might be avoided. The generally uniform behavior of the crops indicated that the injury could not be attributed to the climatic conditions of a single season. Chemical analyses accompanying more recent reports concerning injury of crops by lime and potash (**8**, **12**, **13**) show that the physiological cause underlying the injurious effect is frequently a disturbance in the

internal mineral nutrient balance so profound as to upset or prevent normal metabolism in the plant. Tissue analyses in such cases are far more valuable than soil analyses in indicating proper fertilizer practice to be pursued. The investigation herein described was undertaken to determine if chemical analyses of crop plants in their vegetative phase would indicate why the fertility of certain poorly productive, acid muck soils was apparently diminished by use of calcium and potassium fertilizers. For purposes of comparison, a soil similar to the above in composition and texture but responding favorably to lime and potash was included in the experiment.

Experimental procedure

Samples of soils known to exhibit marked response to lime and potash amendments were thoroughly mixed with finely powdered, chemically pure calcium carbonate or potassium chloride in the proportions indicated in accompanying tables (II, III, IV). 3200-gm. portions of each soil were placed in glazed earthenware pots and 800 cc. of freshly distilled water were added. Yellow Dent corn, Marquis wheat, and Mammoth Red Fancy clover were grown in duplicate pots on treated and untreated (check) soils in a greenhouse at 60° C. The weight of soil plus water in each pot was readily maintained at 4 kg. by daily replenishment of water lost in transpiration and evaporation, since neither was high. Plants were harvested for analysis at the end of the tenth week by removing entire pot contents to a large pan in which the roots were washed free of soil with distilled water. After rapid drying on filter-paper to remove excess water, the entire crop from each pot was weighed, minced and the pulp thoroughly mixed. Duplicate samples were retained for moisture determinations and the remainder of the pulp pickled for storage in boiling 80 per cent. alcohol containing 2 gm. calcium carbonate per liter. Preceding analysis, alcohol was evaporated and samples dried to constant weight in a vacuum oven at 80° C.

Four distinctly acid organic soils differing in their response to fertilizers were employed. The first was a black soil low in potash and light in weight responding favorably to lime but unfavorably to potash. The second soil was a brown, sandy, low lime muck, exhibiting injury following both lime and potash applications. The third was a black, light weight, powdery, low potash muck showing injury to cereals following use of lime but responding favorably to potash amendments. The fourth was a friable, black muck responding favorably to both lime and potash. Chemical analyses of the untreated soils are given in table I.

TABLE I

CHEMICAL ANALYSES OF FOUR UNTREATED MUCK SOILS

	PERCENTAGE DRY WEIGHT			
	SOIL 1	SOIL 2	SOIL 3	SOIL 4
Total calcium	1.67	0.08	3.44	3.54
Total magnesium	0.18	0.10	0.40	1.02
Total iron	0.10	2.02	1.21	2.22
Total potassium	0.30	1.52	0.29	1.05
Total phosphorus	0.34	0.08	0.19	0.20
Total sulphur	0.44	0.11	0.54	0.20
Total nitrogen	3.90	1.42	3.62	1.22
Volatile matter	83.00	42.61	48.10	47.78
Lime requirement, ppm.	2700.00	3500.00	2700.00	1200.00

Analytical methods

Lime requirements were determined by the VEITCH method and mineral content by official methods (**2**). Entire plants including roots were analyzed. Organic nitrogen was determined by the GUNNING method and was modified to include nitrates in determination of the total nitrogen content of tissues. Nitrate nitrogen was determined by difference. After clarification with neutral lead acetate, soluble sugars in an aliquot of alcoholic filtrate were hydrolyzed with concentrated hydrochloric acid and analyzed volumetrically for total reducing sugars by the modified BERTRAND permanganate method. Copper values are expressed as glucose determined from MUNSON-WALKER tables (**2**). Total carbohydrates include the polysaccharides of the dry residue (F_3 fraction) calculated as starch and determined by direct acid hydrolysis, plus the total soluble sugars of the filtrate (F_1 and F_2 fractions).

Moisture content is expressed as percentage of wet weight. Portions of original moisture samples were ignited and used for determination of minerals according to official methods. Calcium was precipitated as oxalate, potassium as cobaltinitrite, and iron as ferric hydroxide, the latter being subsequently reduced with zinc in concentrated sulphuric acid. All three were then titrated against standard potassium permanganate. Magnesium was determined gravimetrically as pyrophosphate. Cryoscopic methods were employed for the estimation of osmotic pressure of freshly expressed sap, the observed depression of freezing points being converted to atmospheric pressures (**16**). Hydrogen ion concentrations were measured potentiometrically.

TABLE II

CHEMICAL ANALYSES OF VICTORY OATS

Salt added (ppm.)	Percentage of dry weight: Iron	Magnesium	Calcium	Potassium	Nitrate nitrogen	Total nitrogen	Total sugars	Total carbohydrates	Sap extract: Osmotic pressure (atm.)	pH	Dry yield (gm.)
Soil 1											
Check	0.067	0.16	0.40	0.47	0.18	2.25	4.51	13.80	5.40	6.0	5.70
400 KCl	0.080	0.08	0.31	0.82	0.26	2.01	5.09	11.41	5.80	5.1	4.12
4000 $CaCO_3$	0.089	0.28	0.57	0.30	0.69	1.70	4.40	10.07	4.25	6.5	3.78
Soil 2											
Check	0.120	0.27	0.35	0.75	0.38	1.74	5.20	9.60	6.30	5.2	4.73
400 KCl	0.140	0.12	0.14	0.90	0.09	1.13	5.69	9.02	6.65	5.0	2.76
4000 $CaCO_3$	0.090	0.30	0.49	0.37	0.97	0.90	5.31	7.52	6.52	5.9	4.20
Soil 3											
Check	0.046	0.33	0.32	0.91	0.21	1.80	3.77	10.32	3.85	4.9	4.88
400 KCl	0.053	0.21	0.42	1.19	0.07	2.33	5.26	12.41	4.81	4.5	5.71
4000 $CaCO_3$	0.082	0.38	0.58	0.46	0.92	1.67	5.20	8.75	3.00	6.2	2.90
Soil 4											
Check	0.081	0.22	0.29	0.74	0.61	1.75	6.46	11.94	4.26	4.7	3.70
400 KCl	0.097	0.20	0.19	0.95	0.40	1.94	7.00	12.49	5.70	4.4	5.10
4000 $CaCO_3$	0.061	0.29	0.26	0.57	0.30	2.07	8.11	13.40	4.92	5.8	4.37

TABLE III

ANALYSES OF MARQUIS HARD WHEAT

Salt added (ppm.)	Percentage of dry weight								Sap extract		Dry yield (gm.)
	Iron	Magnesium	Calcium	Potassium	Nitrate nitrogen	Total nitrogen	Total sugars	Total carbohydrates	Osmotic pressure (atm.)	pH	
Soil 1											
Check	0.021	0.24	0.72	0.28	0.20	2.27	6.85	12.93	7.24	4.6	5.78
400 KCl	0.028	0.20	0.39	0.70	0.47	2.07	6.90	9.66	10.13	4.2	4.48
4000 $CaCO_3$	0.026	0.24	0.50	0.20	0.80	1.40	4.90	10.14	9.90	6.0	3.39
Soil 2											
Check	0.140	0.36	0.41	0.74	0.85	0.99	5.24	14.58	8.87	4.6	5.80
400 KCl	0.145	0.28	0.27	0.90	0.29	0.64	4.47	8.77	9.00	4.7	3.20
4000 $CaCO_3$	0.117	0.39	0.65	0.44	0.70	0.90	4.97	10.40	9.22	6.0	5.10
Soil 3											
Check	0.014	0.17	0.32	0.78	0.10	0.76	6.88	14.21	7.26	4.9	5.47
400 KCl	0.029	0.20	0.24	0.89	0.00	0.94	5.98	13.60	7.40	4.5	6.72
4000 $CaCO_3$	0.009	0.31	0.38	0.54	0.56	0.70	3.94	9.95	6.05	5.7	4.28
Soil 4											
Check	0.121	0.36	0.38	0.67	0.74	0.79	4.44	13.46	7.27	5.0	4.87
400 KCl	0.165	0.32	0.18	0.82	0.40	1.35	6.12	14.80	9.03	4.7	3.96
4000 $CaCO_3$	0.042	0.40	0.51	0.48	0.71	1.30	6.78	14.05	10.03	5.8	4.80

TABLE IV

ANALYSES OF YELLOW DENT CORN

Salt added (ppm.)	Percentage of dry weight								Sap extract		Dry yield (gm.)
	Iron	Magnesium	Calcium	Potassium	Nitrate nitrogen	Total nitrogen	Total sugars	Total carbohydrates	Osmotic pressure (atm.)	pH	
Soil 1											
Check	0.014	0.20	0.46	0.70	0.17	2.45	4.46	12.20	8.61	5.1	9.88
400 KCl	0.020	0.14	0.15	0.81	0.62	1.97	6.12	10.74	9.41	4.3	8.10
4000 $CaCO_3$	0.042	0.24	0.78	0.56	0.41	1.70	4.87	9.26	8.58	5.6	6.70
Soil 2											
Check	0.096	0.25	0.35	0.40	0.18	2.26	6.04	10.85	7.22	4.8	7.90
400 KCl	0.127	0.18	0.20	0.72	0.26	2.02	4.00	9.37	9.46	4.3	4.96
4000 $CaCO_3$	0.141	0.27	0.84	0.31	0.42	1.74	5.71	8.74	8.07	6.2	6.40
Soil 3											
Check	0.036	0.27	0.44	0.52	0.23	2.06	5.14	12.12	6.42	5.6	8.26
400 KCl	0.045	0.20	0.28	0.89	0.14	2.46	7.28	14.71	9.21	4.7	9.98
4000 $CaCO_3$	0.071	0.38	0.71	0.36	0.19	1.85	4.66	9.12	8.89	6.1	6.02
Soil 4											
Check	0.091	0.32	0.37	0.74	0.30	2.17	6.10	13.11	8.80	4.8	4.59
400 KCl	0.093	0.20	0.25	0.90	0.11	2.47	5.38	13.64	11.02	4.2	7.04
4000 $CaCO_3$	0.070	0.40	0.52	0.58	0.21	1.85	4.88	12.09	9.75	5.8	7.12

Discussion

The response of each of the three crops to a given fertilizer was quite consistently the same for each soil. High yields were as a rule associated with more rapid growth of the plants and generally improved appearance, involving more abundant and healthier foliage, stiffer stems, and greater stature. External evidences of injury, on the other hand, were stunted growth and procumbent or spindly habit associated with abnormal, often chlorotic or spotted leaves. Only on soils which had been treated with potassium chloride did plants develop stiff and erect stems, from which it may be inferred that abundance of potassium is more essential than lime for development of normal stem habit in grain crops grown on these soils. Microscopic examination of tissues from recumbent stems disclosed weak sclerenchyma cells with large lumina and poorly developed thickenings in tracheae, conditions suggesting disturbed carbohydrate metabolism especially with reference to polysaccharides.

The analytical data show that disturbed carbohydrate metabolism was coincident with reduced potassium content which, in the case of plants grown on the first three soils, became low enough to create a potash insufficiency, if yield and symptoms of injury be taken as criteria. That potassium is important in carbohydrate storage has long been known, though its precise mode of action is not yet clear. In an earlier report (**27**) it was noted that potassium may also be important in protein synthesis since the presence of abundant carbohydrates and inorganic nitrogen appear to favor protein accumulation (**35**). Accumulation of nitrates in high lime tissues may be due to the fact that lime increases soluble soil nitrates (**9, 32, 34**) which are absorbed by the plant but not converted to proteins because, potash being deficient, there are insufficient carbohydrates to combine with the large store of nitrates. Aqueous soil extracts analyzed for mineral nutrients indicated that there was soluble potassium present which presumably was available for absorption. The addition of alkaline fertilizers to soils is known to liberate other adsorbed bases and for this reason the quantity of potassium in the soil solution would be expected to increase rather than to diminish as a result of liming. Yet this is not necessarily the case for highly colloidal soils as deficient in minerals as those here considered. Many soil filtrates showed marked hysteresis, being initially acid to indicators but later becoming alkaline, a behavior not uncommon in extracts of organic soil (**36**). This phenomenon probably explains why duplicate analyses of organic soil solutions give inconcordant results and it probably also influences to a considerable extent the availability of plant nutrients in such soils. It is also necessary to point out in this connection that during absorption the selective permeability of root hairs comes into

play and the rate at which they take up soluble ions is not necessarily proportional to the concentrations of these in soils. Whatever may be the conditions controlling absorption of mineral nutrients from these soils, the fact remains that many plants on high lime soils showed external and internal symptoms of potash hunger apparently induced by the lime employed to correct acidity. Mature leaves were elongate, somewhat flaccid, dull in color with slightly wavy margins and puckered surfaces. Young leaves were distinctly yellowish. Some of these symptoms of potash insufficiency are similar to those previously observed in tobacco grown on sandy soils subjected to leaching (**12**).

The above described calcium-potassium balance of lime-injured plants does not fully account for the chlorotic condition of their young leaves, a symptom ordinarily associated with iron insufficiency. It was consequently at first difficult to reconcile the high iron content of these plants with their chlorotic appearance. The explanation of this apparent anomaly was suggested by the fact that the hydrogen-ion concentration of the sap of plants from limed soils was considerably lower than that of plants from unlimed soils, a condition apt to be of the utmost importance in controlling iron solubility (**3**, **22**, **37**) and absorption (**17**, **30**). Microchemical tests for iron were made on tissues (**41**) to determine if reduced sap acidity interfered with iron translocation. These tests disclosed large amounts of iron in the nuclei of root hairs and the vascular elements of roots and basal stem nodes. The root hairs appeared healthy but there was evidence of obstruction by iron salts in many root tracheae and sieve tubes. Sections from higher stem nodes gave positive tests for iron in a few sieve tubes and companion cells while leaf sections, with the exception of a few from mature leaves, gave tests for traces or were entirely negative. High lime plants generally displayed this peculiar iron immobility, characterized by copious precipitation in roots with a tendency to diminish in aerial parts to such an extent that leaves displayed iron chlorosis. The chemical analyses here given (tables II, III, IV) disclosed no irregular distribution of iron because shoots and roots were not analyzed separately. Lime, then, not only created potash insufficiency but also reduced sap acidity to the point of interference with internal iron mobility.

Marsh and Shive (**28**) working with soy beans in solution cultures have pointed out that solubility of ferric salts, both in culture solutions and in sap, diminishes rapidly as neutrality is approached. Only when the sap is distinctly acid do adequate amounts of iron absorbed by roots actually reach the leaves. These investigators found maximum yields correlated with small quantities of iron evenly distributed in aerial parts of plants grown in moderately acid media. Availability of iron was correlated with

its solubility, and tolerance of the tissues to it was controlled by the nature of the culture solutions employed. Highly acid media were injurious due to iron toxicity. It is to be pointed out that the iron content of lime-injured tissues herein reported runs higher than that reported by MARSH and SHIVE. This is due to the fact that they rejected roots which were covered with precipitated iron and analyzed only tops which were low in iron as already explained.

There are also a number of other cases on record in which lime and the concomitant diminution of acidity reduced iron solubility to the detriment of plant growth. MAZÉ (**29**) induced chlorosis in corn and found that it might be due to deficiency of sulphates as well as iron in plants. Lime injury involving chlorosis has been reported for pineapples by GILE (**13**), for rice by GILE and CARRERO (**14**), for pears by MILAD (**31**), and for citrus fruits by LIPMAN (**26**). In spite of chlorotic conditions in the tops, accumulation of iron may sometimes become so great in roots and basal nodes as to be toxic, either directly or as a result of over-stimulating oxidase activity (**18**). HOPKINS and WANN (**20, 42**) find that iron is removed by adsorption on calcium phosphate which gradually precipitates as solutions become alkaline, a physico-chemical effect capable of influencing iron availability within the plant as well as in culture media. The effect of tissue colloids in controlling succulence, hardiness and imbibition is well understood and they are also undoubtedly important in nutrient intake (**24**). It has been noted by CHANCERL (**4**) and HANSTEEN-CRANNER (**15**) that calcium has a tendency to accelerate transpiration. In plants, a portion of whose tracheae have become obstructed by precipitation of iron, water loss may exceed the rate of replenishment from below, thus creating a marked saturation deficit in leaves and causing flaccidity. It will also be noted from the tables that osmotic pressures of sap were lower in plants on limed soils, indicating a general diminution of soluble materials as a consequence of calcium applications. In fact, it is possible that lime or alkalinity resulting from it interferes not only with the mobility of iron but with the mobility of other solutes as well.

The fact that lime applications proved beneficial on soil 4, in which iron and magnesium were relatively abundant, seemed attributable to the fact that iron toxicity was allayed and intake of iron reduced though not to the extent of inducing chlorosis, because leaf xylem and border parenchyma gave clearly positive microchemical tests for ferric ions. Untreated cultures of this soil produced plants with narrow, green leaves, speckled with brown and showing a tendency to turn yellow and die at the tips. This soil also responded favorably to potash amendments but, though crops improved in general vigor and in yield, small brown specks were still dis-

cernible in the leaves showing persistence of iron toxicity. Crops on this soil were benefited most by a combination of calcium carbonate and potassium chloride. Soil 4 was the least acid of those investigated, yet it is doubtful if acidity as determined by ordinary methods can be considered an index to the lime requirement of these or other highly colloidal soils (**43**, **44**).

Plants in the untreated cultures of soil 1 were stunted and produced small, smooth, flaccid, generally pale yellow, mottled leaves with green patches near the midrib, all of which symptoms were intensified by use of potash. Stems, however, were fairly stiff and erect. Even though sap acidity and iron content increased following use of potash, there was no evidence of iron toxicity, probably because the soil was too low in this element to permit excess absorption thereof. The symptoms of injury coupled with the results of plant analyses suggest that the explanation for the injurious effect of potash in the case of this soil lay in the fact that its increase in the tissues was made at expense of both lime and magnesium. In fact, it will be noted from the accompanying tables that there was a rather regular diminution of both calcium and magnesium in plants following use of potash, though magnesium hunger did not become apparent in all cases. To deficiency of calcium may be attributed the poor development of foliage, but coupled with this in producing the stunted condition of the plants may be disturbed root activity, since root hair production is known to be largely dependent upon an adequate supply of calcium (**5**, **10**, **40**).

KELLEY and CUMMINS (**23**) have reported low calcium concomitant with high potassium content of citrus leaves, in which mottling develops when calcium becomes insufficient. GARNER and others (**12**) have observed depression of lime and magnesium in tobacco, often to the degree of severe injury, following use of potash fertilizers. The characteristic symptoms of the injury called "sand drown" they found to be due to magnesium starvation. The tips of lower leaves first became chlorotic and from that point chlorophyll decomposition continued progressively until only the veins remained green. These investigators found use of potassium sulphate and heavy doses of potassium chloride responsible for the disease, at first thought due to heavy rains. Magnesium insufficiency, accentuated by use of potash, was apparently the reason for the unproductivity of soil 1, as it responded very favorably to applications of a mixture of dolomitic limestone with potassium chloride. As stated above, the unfavorable effect of lime alone on this soil was attributable to interference with internal mobility of iron.

On soil 2, potash injury was characterized by the chlorotic condition of young leaves which never became large nor very turgid. Older leaves

possessed a distinctly dull color and showed in addition to the characteristic yellow-green chlorophyll mosaic described above, numerous small brown specks indicative of excess iron. Soil 2 was relatively high in iron (table I) and crops grown on it showed a marked increase in absorption of iron following use of potash. A combination of dolomitic limestone and potash proved only slightly beneficial to this soil in spite of the fact that it was extremely low in magnesium, the failure to respond to this treatment being due apparently to the fact that iron remained toxic.

On soil 3 the response of crops to potash amendments was quite favorable. The potash-treated soils produced healthy, erect stemmed plants with large, deep green leaves, the potassium materially increasing the stiffness of the stem. Though increased potassium absorption resulted in somewhat smaller intake of lime and magnesia, the diminution in amount of these was not great enough to induce symptoms of calcium or magnesium hunger. The increase in soluble iron correlated with increased acidity was obviously beneficial to the plants because their green color was considerably intensified. Plants on this soil showed the greatest increase in osmotic pressure following potassium treatment, a response which was noted in the case of plants from the other soils but to a less marked degree. Potassium salts were found by GARNER and his collaborators (**12**) to increase the osmotic concentration of sap in tobacco, the rise in concentration being due to the greater concentration of electrolytes. When no potassium fertilizers were used, most of the osmotic components of tobacco sap were soluble organic constituents, probably carbohydrates.

The above described variations in mineral nutrient content were correlated in fairly definite ways with organic products in the plants investigated. Injury by lime or potash was in general marked not only by increases in nitrates but also by reductions in organic nitrogen and carbohydrates. High yields, on the other hand, showed generally reversed conditions. The fact that high-lime, low-potash plants show carbohydrate decrease coupled with nitrate increase may be taken to indicate that carbohydrates are insufficient to combine in proper amounts with inorganic nitrogen in protein formation. In these instances, lime applications have diminished potash content to the point of starvation which interferes with normal carbohydrate metabolism. The effects of potash starvation are accentuated by the disturbed iron mobility which induces chlorosis of tops in lime-injured plants. That is, lack of iron minimizes the photosynthetic area in leaves and lack of potash interferes with carbohydrate storage. If potassium is essential for the synthesis of carbohydrates and inorganic nitrogen into proteins, its deficiency in lime-injured plants is another reason for their low organic nitrogen content.

Potash injury, though resulting in reduction of carbohydrate and organic nitrogen content, operated in an indirect manner, apparently by increasing sap acidity which often favored iron toxicity. In the case of soils 1 and 2, magnesium absorption was so depressed that symptoms of its insufficiency also became apparent, and in these instances magnesium chlorosis was associated with excess soluble iron in interfering with photosynthesis. Consequently, the ultimate result of injury by potash or lime was reduction in the amount of carbohydrates and organic nitrogen. High yields were in general correlated with more nearly balanced conditions among mineral nutrients, and with high carbohydrate and protein content.

The great variability in concentration of lime and potash in the plants grown on these muck soils is in part attributed to the highly colloidal nature of the soil particles, which on the one hand prevent leaching of nutrients but on the other may create physiological deficiency thereof by adsorption. Injury from a given fertilizer to plants on colloidal soils may be direct or it may be due to toxic substances liberated by replacement. Mineral fertilizers added to organic soils may depress the solubility of elements present only in small quantities and thereby make them unavailable to plants. For these reasons chemical analyses of unproductive muck soils give little clue to the proper fertilizer practice to be pursued in improving them. In such instances plant analyses indicate in a more satisfactory way the mineral nutrients required, at least for the early vegetative phase of the grain crops here reported. Fertilizers needed during maturation stages would probably be different from those required in early development due to the differences in metabolism during vegetative and reproductive phases in the same species (**6**, **25**, **33**).

Summary

Grain crops on acid muck soils low in potash are often injured by additions of calcium carbonate, which may entail potash hunger and consequent interference with carbohydrate storage, or induce chlorosis by making sap too alkaline to maintain iron in solution. Additions of potassium chloride to such soils may prove injurious to grain plants by increasing iron accumulation in the tissue to the extent of toxicity, or by reducing lime and magnesium content to the point of starvation. Sap is more acid in high potash than in high lime tissues. Soil and sap acidity favor iron solubility and absorption. High yield in young grain plants was associated with high carbohydrates and high organic nitrogen. Low yields were characterized by low protein, low carbohydrate, and high nitrate content.

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AN APPARATUS FOR THE GROWTH OF PLANTS IN A CONTROLLED ENVIRONMENT[1]

A. R. DAVIS AND D. R. HOAGLAND

(WITH SIX FIGURES)

Introduction

The vagaries of an uncontrolled environment are responsible for many of the conflicting results and conclusions found in publications dealing with plant growth. It is common knowledge that workers in different institutions, and indeed often in the same institution, find great difficulty in duplicating the findings of one another, not on account of inadequate description of methods, but because some environmental influence was not controlled. This is particularly true where yield is the criterion upon which the interpretation is based. We have in mind as an example a comparison of the growth obtained in two diverse types of culture solutions.[2] Wheat grown in them under natural conditions of illumination and temperature during various months of the year gave yields significantly the same during the winter months and significantly different during the summer period. One's conclusions in such a case would obviously depend upon the time of year the observations were made. Again in a more recent investigation, the data for which are given in table I, similar results are apparent.

TABLE I

THE INFLUENCE OF SEASONAL SOLAR ILLUMINATION ON YIELD OF LITTLE CLUB WHEAT. TEMPERATURE 20.5° C.

PERIOD	NOV. 22/26 DEC. 20/26	DEC. 29/26 JAN. 27/27	FEB. 18/27 MAR. 18/27	MAR. 25/27 APR. 22/27	MAY 2/27 MAY 30/27	AUG. 17/27 SEPT. 13/27
	cgm.	cgm.	cgm.	cgm.	cgm.	cgm.
Mean dry weight 100 tops	25.7	15.2	39.9	46.2	97.5	86.9

In this last experiment the same lot of pure line seed was used throughout and germination conditions, temperature, culture solutions, etc., were maintained as nearly identical as possible in the several series. The chief

1 The authors wish to acknowledge the generous grants made by the University Board of Research, without which this work could not have been pursued.

2 Unpublished data.

variable was illumination and it was sufficient to cause a fluctuation in yield from 15 cgms. in December to 97 cgms. in May! When an environment is characterized by such conditions of change the duplication of yields becomes a chance achievement and conclusions drawn from them may frequently be just as much a matter of guesswork. Such data as these indicate the necessity of conducting plant experimentation, whenever possible, under controlled conditions. The mere recording of climatic fluctuations is insufficient because of the difficulty of isolating the effect of a single variable.

The construction and maintenance costs of equipment rather than obstinate mechanical problems or a failure to realize the importance of this method of attack ha~~s~~ve prevented most plant physiologists from taking the steps necessary toward control of environment. One has in mind more particularly the extensive equipment in use at the Boyce Thompson Institute (**1, 2**). Such equipment is highly to be desired, permitting as it does the study of plant growth over a wide range of conditions adequately controlled by dependable instruments. It would be most unfortunate, however, if failure to finance a similar installation should discourage the worker who has a limited budget at his disposal. There are many problems which may be attacked just as efficiently with a small and relatively inexpensive control equipment such as the one employed by Tottingham (**10**) at the University of Wisconsin, that of Hottes at the University of Illinois, or the one we wish to describe in this paper.

We have had in mind an apparatus that would permit efficient control of the environment, would not require a large maintenance fund, and at the same time would meet requirements for expansion both with respect to budget and research program. The apparatus now in use in our laboratory is the result of several years experience and while still in a state of development, it is meeting the demands placed upon it in a highly satisfactory manner. Moreover it contains, we feel, certain essential elements of design which will make an appeal to workers attacking diverse physiological problems in botany and in the allied fields of plant pathology, horticulture, agronomy, etc. It must be borne in mind that this apparatus is not designed for extensive growth investigations where large numbers of plants are involved and where soil is employed as a culture medium. It is rather for the intensive study of plants easily handled by the water culture method. The latter makes for economy of space and permits control of the source of inorganic ions. The equipment is peculiarly adapted to the study of nutrient solution effects in a controlled environment; studies on the influence of light intensity and exposure time; light-temperature relationships; the effect of differentials in temperature between roots and tops;

humidity effects; changes in the gaseous components of the atmosphere; factors influencing the production of organic compounds, etc., etc. These are but a few examples of the many problems which will at once occur to the reader. With wheat as the plant under investigation it has been possible to grow as many as 100 plants for a period of four weeks in each chamber and as is indicated in table II, this will give a mean of unusual reliability.

There are two important tests of such an apparatus. First, the plant grown must compare favorably in appearance and composition with that obtained under favorable conditions in nature, and second, there must be the possibility of reproducing environments and as a consequence replicating results. We have found our apparatus extremely satisfying in both these respects. As far as we have been able to determine by superficial appearance and by chemical analyses, illumination has been satisfactory both as to intensity and quality. The wheat plants have been green, vigorous, have tillered well, and have shown etiolation only when illumination was deliberately decreased. These points are illustrated photographically in fig. 1, where the two cultures on the left, grown under artificial light in

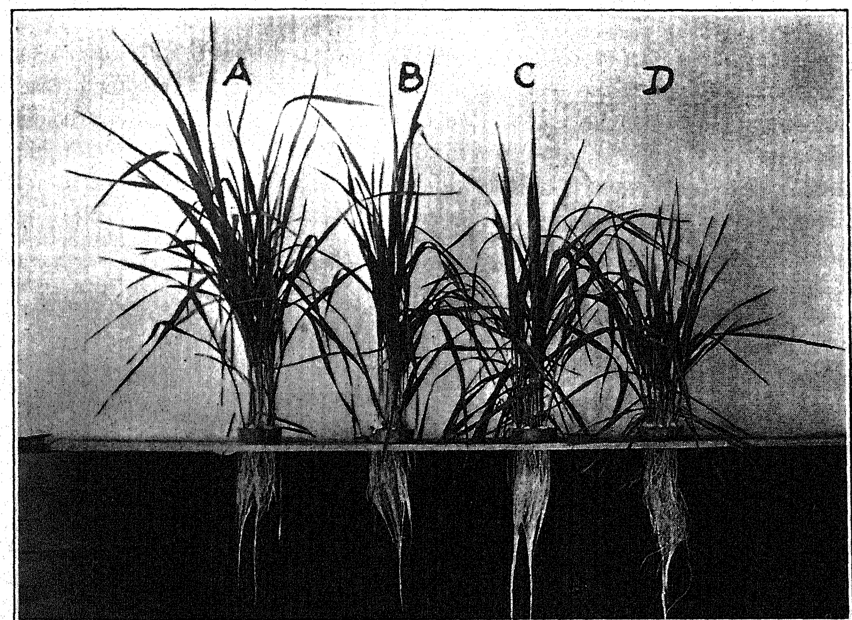

FIG. 1. Comparison of the effect of artificial and solar illumination on growth of wheat during March. A = 2610, B = 1640 foot candles electric light, 16 hours per day at 20.5° C. C and D, full solar illumination. C = 20.5° C. and D = temperature uncontrolled. Plants 28 days from transplanting.

a controlled environment may be compared with those on the right grown at the same temperature under solar illumination during March. A still more striking comparison is shown in fig. 2, where the culture on the left was subjected to solar illumination during December at 20.5° C., the one in the center and on the right to artificial light, the first at 17° C., the second at 20.5° C.[3]

As a general statement we may say that wheat plants grown in a controlled environment at 2600 foot candles artificial illumination, 16 hours daily exposure time, and at 20.5° C. exceeded in yield those of parallel series grown in a greenhouse under solar illumination and at the same controlled temperature for any month of the year with the exception of May. During this month natural light conditions were especially favorable and approximately equivalent yields were obtained. The above statements have reference to 28-day plants only. However, we have demonstrated to our own satisfaction that, although considerably hastened, normal maturation of the plant can be effected in such a controlled environment. The two mature Little Club wheat plants shown in fig. 3 show normal tillering, heading and seed formation, the heads being above average for field conditions.[4] The plants averaged seven heads, each maturing 50–60 well filled kernels, these latter being 30 per cent. heavier than the parent seed. The increased weight was probably due to a higher nitrogen content. Previous investigators have reported the maturation of wheat under continuous artificial illumination, HARVEY (**2**), MAXIMOW (**6**), and BAKHUYZEN (**11, 12**) among others. HARVEY reports the development of a single head with "6–8 well filled seeds" where the illumination was continuous and of 400–800 foot

[3] The plants shown in figs. 1 and 2 were grown for 28 days in two quart Mason fruit jars containing 1850 cc. of solution. Five plants were grown in each jar. The solution employed was one which has been used in this laboratory over a period of years and for many different plants. The elements are supplied as $Ca(NO_3)_2$, KNO_3, KH_2PO_4, $MgSO_4$, HBO_3 and $MnSO_4$ in the following parts per million concentration:

ION	NO_3	PO_4	SO_4	K	CA	MG	B	MN
Ppm.	700	10	200	190	172	52	0.5	0.5

The plants grew rapidly and the iron requirements were relatively high. PO_4, added from time to time, was maintained at the above low level in order to facilitate iron absorption, otherwise there was no addition of elements during the period noted. K and NO_3, the two ions most rapidly removed, were present in amounts sufficient for the growth period under the conditions imposed. Fe was added as ferric tartrate, 2 cc. of a 5 per cent. solution per jar as needed.

[4] According to Prof. W. W. MACKIE, Division of Agronomy, University of California.

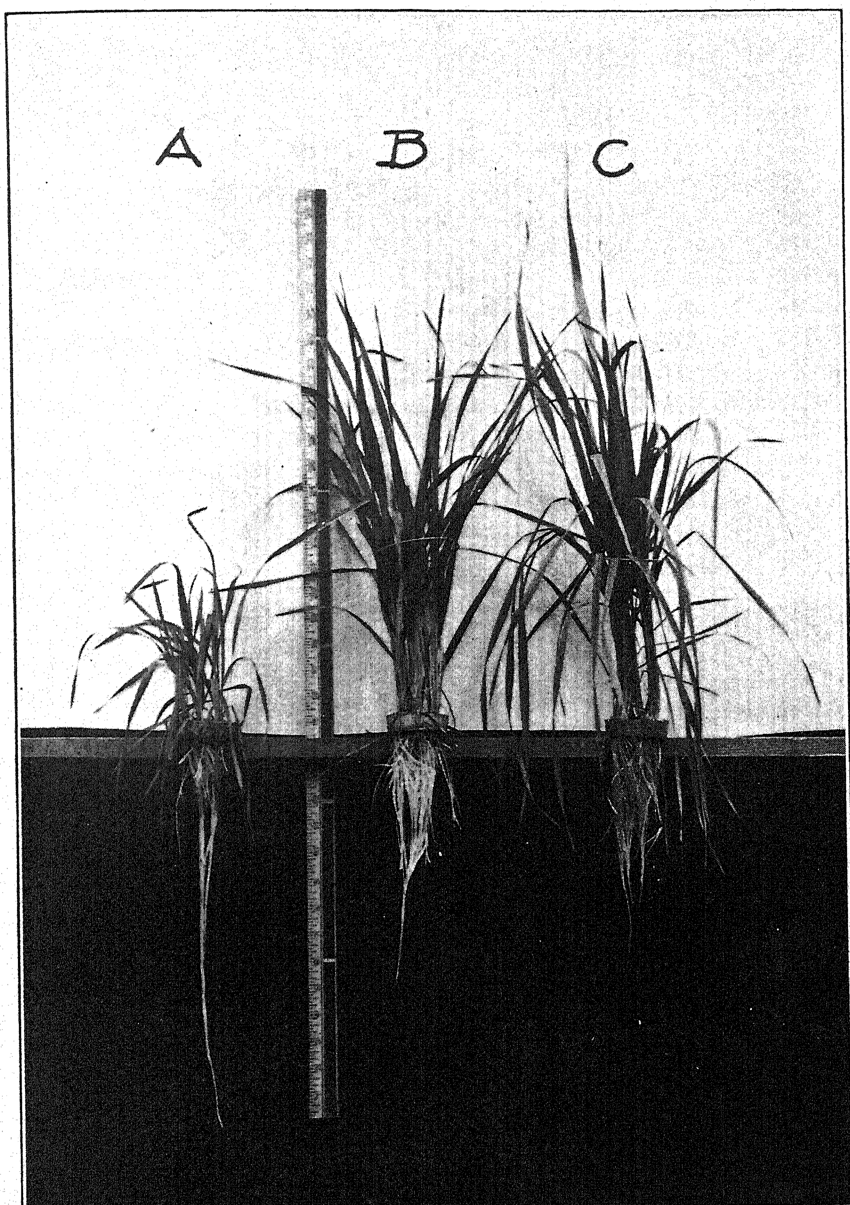

FIG. 2. Comparison of the effect of artificial and solar illumination on the growth of wheat during December. A = full solar illumination 20.5° C. B and C = 2610 foot candles electric light, 16 hours per day, B at 17° C. and C at 20.5° C. Plants 28 days from time of transplanting.

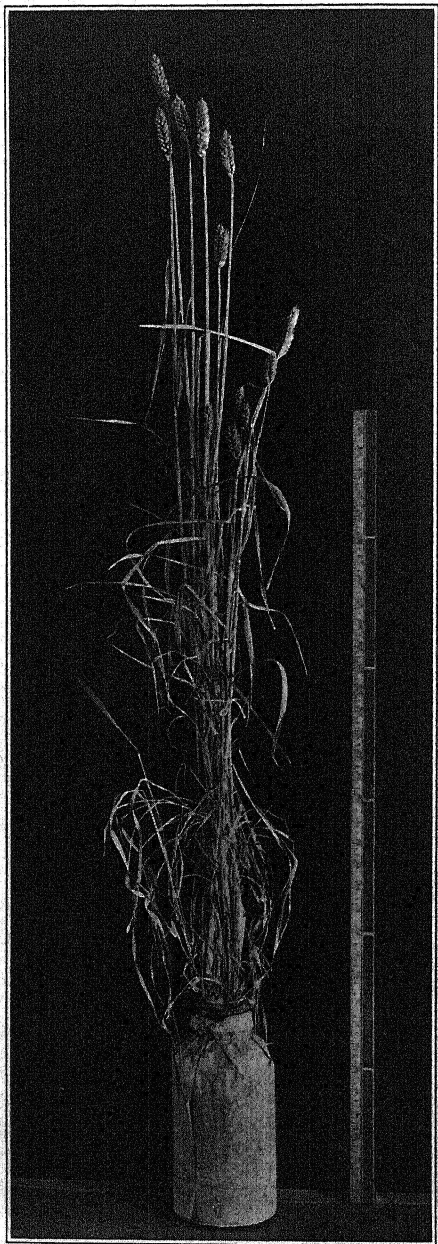

FIG. 3. Two plants Little Club wheat 13 weeks from time of planting. Artificial light = 2610 foot candles, 16 hours per day, 20.5° C.

candles intensity. MAXIMOW does not state the number of heads and seeds formed, nor mentions the appearance of the seeds. BAKHUYZEN has grown Hard Federation to maturity under continuous electric light, one stalk only being permitted to develop. The head and seed formations were stated to be normal.[5]

Duplication of yields has likewise been achieved with a high degree of satisfaction as is apparent from the data in table II. Here the mean dry weight of 100 tops shows a remarkable uniformity and the probable error of the mean (approximately one per cent.) is unusually low. Calculations based upon this probable error indicate less than a 30:1 chance that the greatest differences involved are significant. These data and others to be reported in a subsequent paper, strikingly indicate the feasibility of controlling the environment and of predicting results. Such predictions are not only possible with respect to height and weight of plants at the end of a predetermined period, the time of first tiller appearance, the final number of tillers, etc., but in addition an equation can be developed enabling

TABLE II[6]

DUPLICATION OF WHEAT YIELDS IN A CONTROLLED ENVIRONMENT
ILLUMINATION, 3600 WATTS (2610 f. c.) 16 HOURS DAILY EXPOSURE PERIOD. TEMPERATURE, 20.5° C. DURATION OF EXPERIMENT, 28 DAYS

EXPERIMENTAL PERIOD	NUMBER OF PLANTS	MEAN DRY WEIGHT TOPS	MEAN NUMBER TILLERS	HEIGHT MAIN STALK
		cgm.		cm.
Dec. 29, 1926 – Jan. 26, 1927	100	92.2 ± 0.94	4.0	59
Feb. 18, 1927 – Mar. 18, 1927	100	90.5 ± 0.90	4.0	60
Mar. 25, 1927 – Apr. 22, 1927	100	89.0 ± 1.00	4.2	57
Oct. 27, 1927 – Nov. 24, 1927	100	89.6 ± 0.95	3.9	59
Oct. 27, 1927 – Nov. 24, 1927	100	90.6 ± 0.90	3.8	58

[5] Several earlier workers have demonstrated the feasibility of substituting artificial for solar illumination. For an historical account of this work the reader is referred to POPP'S (7) excellent summary.

[6] In these series a pure line strain of Little Club wheat obtained through the courtesy of Professor W. W. MACKIE, of the Division of Agronomy, this University, was used. The seeds were soaked in the above culture solution for 16 hours at 20.5° C., then distributed on tinned wire netting over pyrex baking dishes containg the same solution. The atmosphere above the seeds was kept saturated with water vapor until the roots were well in contact with the culture medium. At this point the cover was removed and the seedlings grown under 600 watts artificial light until they attained an average height of 9 ± 5 cm. Only those falling within these limits were selected for transplanting.

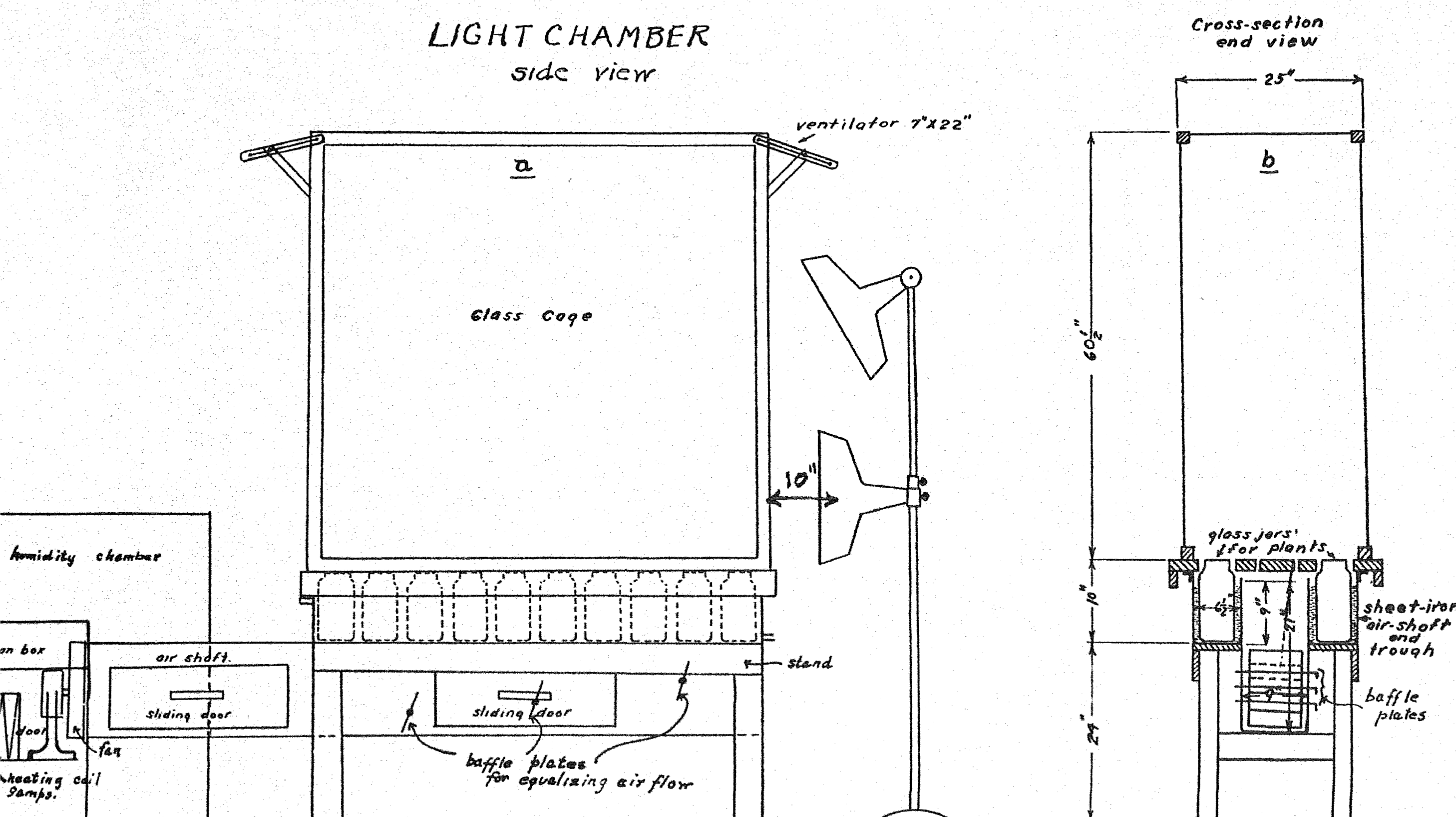

FIG. 4. Apparatus for the control of environment. Side view.

one to predict yields for varying daily exposure times where the intensity of light remains constant. Thus with all the major influences in the environment controlled, one is in a position to study one plant intensively and to learn much of the part played by each portion of the environment at each stage of growth. Moreover, such control and duplication of results enables one to definitely establish a "standard plant" as a reference point.[7]

Apparatus

The apparatus is constructed in units, two of which have been in operation during the past year and a half (figs. 4, 5, 6). Each unit is housed in a white enamelled room, 12 x 12 x 12 feet, provided with bottom ventilators as shown in fig. 5. In addition to these latter, a large opening in the

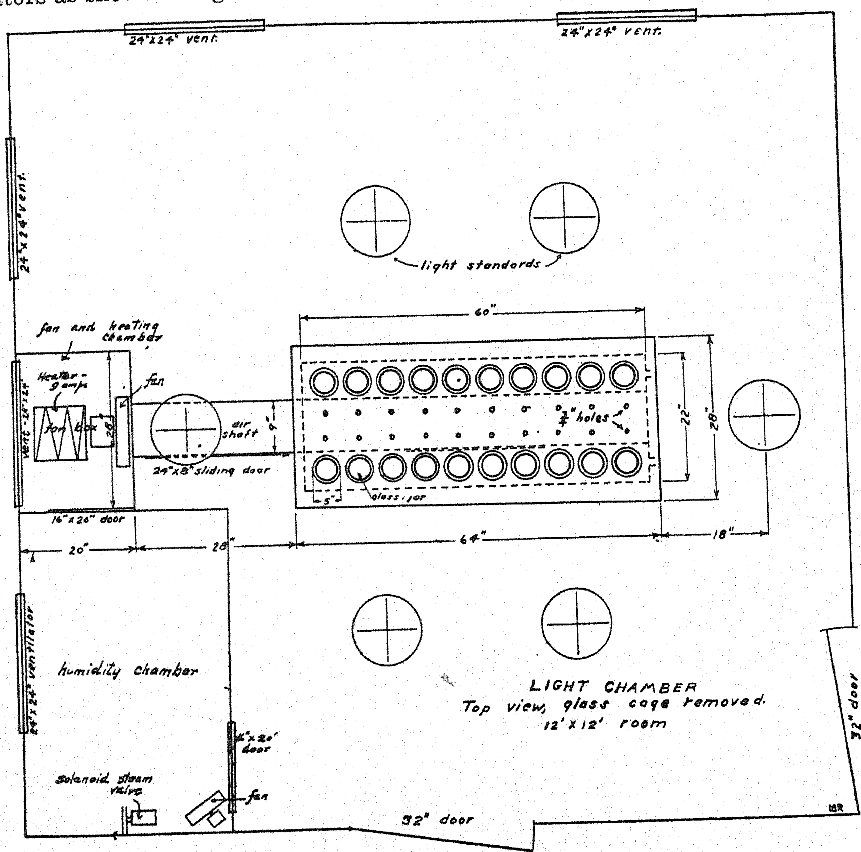

FIG. 5. Apparatus for the control of environment. Top view.

[7] The desirability of plant experimentation under these conditions has been frequently emphasized and is generally admitted. Among others, TREALEASE and LIVINGSTON (9) had this in view in their report on the relation of climatic conditions to the salt-proportion requirements of plants, and again LIVINGSTON (5) in his survey of recent advancements in plant physiology.

ceiling of the room permits an excellent natural draft which may be increased by the use of electric fans. Exclusive of lighting, heating, and humidity control, which will be described separately, the apparatus consists of a plant chamber and its accessories as follows: (a). perforated wooden platform upon which the glass chamber rests; (b). temperature and humidity chamber housing fans, heating and humidity apparatus; (c). air tunnel leading from the temperature-humidity chamber to the plant chamber; (d). galvanized iron troughs upon which the wooden platform rests and which holds the culture solution jars.

Plant chamber

The plant chamber in which the cultures are placed (fig. 6) is of high grade glass (not plate) 5 x 5 x 2 feet, open at the bottom where it rests on the wooden platform and counterpoised over pulleys in the ceiling of the room to permit raising with a minimum of effort. The wooden platform resting on the galvanized iron troughs and air tunnel is pierced with twenty 5-inch holes for culture jars as well as a number of 1-inch holes to facilitate the flow of air from the air tunnel through the plant chamber (figs. 4b, 5). The air tunnel, occupying a space between the troughs and below the platform, leads to the temperature chamber where is placed a 12-inch electric fan and an electric heating unit (figs. 4a, 5). Air flows along the air tunnel, through the perforations in the wooden platform into the plant chamber, thence out through vents at the top of the latter (fig. 4a). The rate of flow through these vents as measured by a "Tycos" anemometer has been established at 400 cubic feet per minute, the equivalent of a gentle but constant breeze over the plants. To equalize air flow in different portions of the plant chamber, adjustable baffle plates are placed in the air tunnel (figs. 4a–b).

Temperature control

The desired temperature is maintained within 1° C. by means of a double relay system controlled by a DeKhotinsky bimetallic thermostat.[8] The first relay, a telegraphic 100 ohm type, is activated by the current from a six volt wet battery; the second relay, a General Electric Co. magnetic switch[9] with twenty-ampere capacity on the secondary, is controlled by the 110 volt A. C. flowing through the secondary of relay no. 1. We have found the two relay system very satisfactory where the amperage required for heating is high since a small current may be sent through the thermostat and first relay and no trouble experienced because of sticking of contact points on the former. As an additional precaution a telephone condenser

[8] Central Scientific Co. No. 13740.

[9] Gen. Elec. C. R. 2610–1265–G 1–1102–60 cyc.

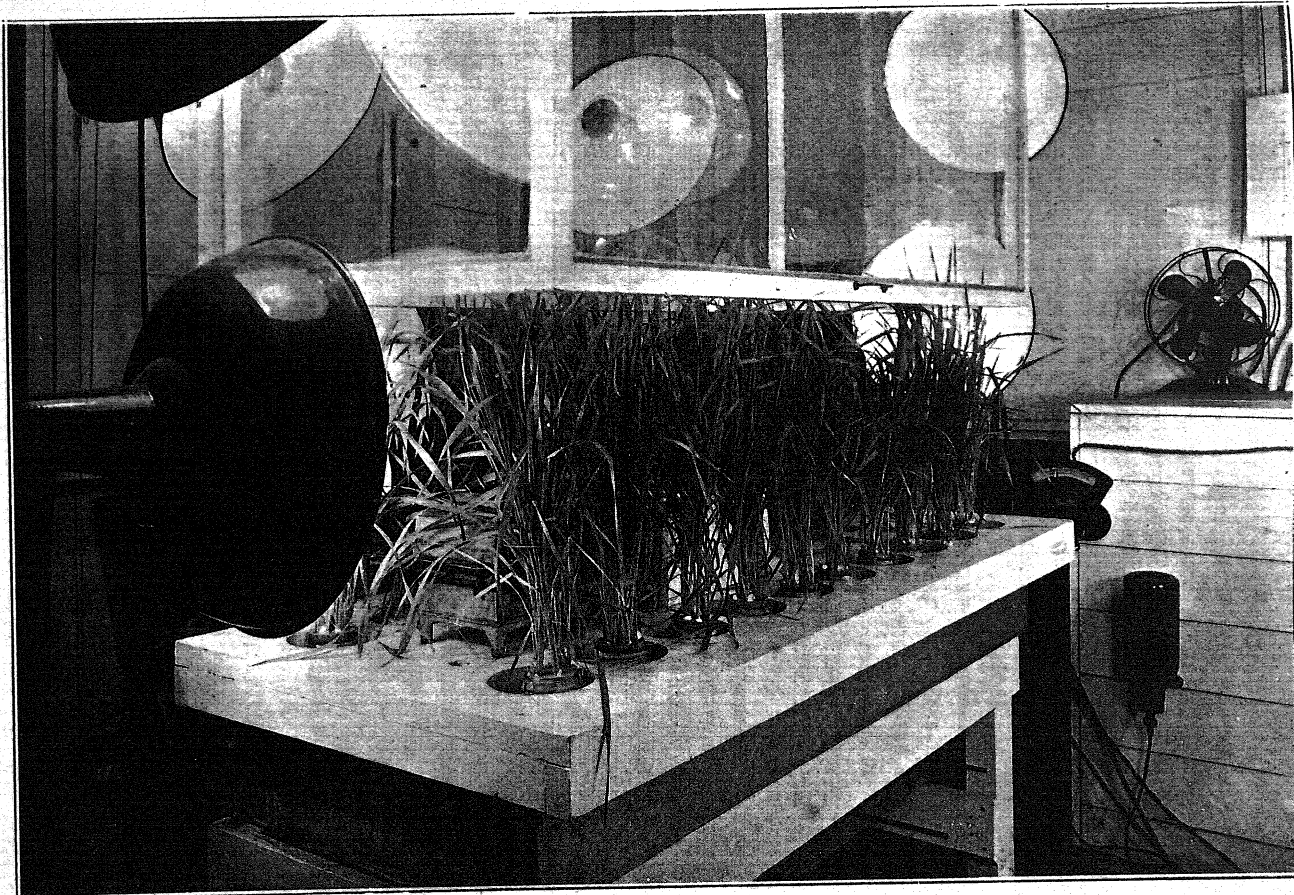

Fig. 6. Apparatus for the control of environment showing 28–day plants grown at 3035 foot candles, illumination intensity, 16 hours per day, 20.5° C. Counterpoised plant chamber partly raised.

is placed across the primary circuit. The heating unit used is homemade and consists of a transite frame 10 x 10 x 10 inches wound with enough no. 24 "Chromel" wire to give it a capacity of 9 amperes. During the cooler months the heat from the lamps is employed as a secondary heating source and by restricting ventilation in the room all or only part of such heat may be utilized. These sources of heat meet the requirements at Berkeley. It is realized however, that in colder sections of the country it may be desirable to pre-heat the air by means of steam or hot water pipes and employ an electric heating unit to give final control. In case a large plant chamber is used and air replacement is relatively slow, steam heat may be used directly, the temperature being regulated by means of a Bristol or similar type controller as is the practice at the Boyce Thompson Institute.

Provision has been made for the maintenance of a differential in temperature between roots and tops, should such be desired. The troughs (fig. 4b) acting as supports for the culture jars are fitted with inlets and outlets so that water of any predetermined temperature can be circulated. This feature precludes the use of a rotating table as employed by TOTTINGHAM (**10**) and by HARVEY (**3**). However, owing to the rather rigid balancing of conditions in different parts of the chamber, we feel such a mechanism is not necessary, although as a precaution against any undetected environmental inequalities, it has been the practice to change the position of the jars at regular intervals. The unusually small probable error of the mean (noted earlier) as well as the uniformity of distribution on a frequency curve indicate no unusual asymmetric influences.

In installations of this sort the disposal of heat from lamps having a maximum emission in the near infra-red presents a problem, the solution of which usually involves the interposing of a water screen between lamps and plants. Although efficient from the point of view of heat absorption, certain secondary difficulties are encountered which tend to decrease the value of the method. The glass water screen must be kept scrupulously clean to prevent a lowering of light intensity in the visible spectrum. Furthermore, unless the correct temperature relation is maintained between glass and air beneath, moisture is condensed on the lower surface of the former. This method, with its pumps and water circuit, adds to the complexity of the equipment and limits one in the distribution of lamps. It was found possible to simplify our original apparatus in which we employed a water screen, by substituting a slow current of air through the plant chamber and a relatively rapid replacement of air in the surrounding room. In the present rooms the excellent natural draft is usually all that is required to keep the plant chamber at a point within two degrees of air at the intake. A refrigeration system would add greatly to the range of experimentation.

ILLUMINATION[10]

The method of heat dissipation described permits the placement of lamps above and on all four sides of the plant chamber as well as at any distance from it.[11] The lamps are on movable standards, adjustable as to height, and are equipped with a heavy cast iron base. The standards, two lamps to each, are distributed two on each side of the plant chamber and in addition two lamps are suspended from the ceiling above. In the experiments cited the filament of all lamps was within ten inches of the glass wall and approximately sixteen inches from the growing plant. The distance of the upper lamp from the plant changed slightly with growth but not enough to significantly affect the illumination values given. The reflectors employed are the dome type[12] eighteen inches in diameter and possess a wide angle of dispersion. The manufacturer's rating gives a reflecting efficiency of 76 per cent.

In all the experiments reported herein unless otherwise noted, we have made use of the 300 watt Mazda C gas-filled lamp, the energy distribution being very similar to that given by POPP (8) for a 1000 watt lamp of the same type. Although these lamps are rated at 1000 hours their efficiency under our conditions began to fall off noticeably after 500 hours both with respect to total energy and energy in the visible spectrum.[13] This latter decrease is more marked because the maximum energy band, which in these lamps lies in the short infra red, shifts still further in the direction of longer wave length with use. This is due to volatilization of the filament resulting in increased resistance, lower consumption of current and decreased temperature. Because of such deterioration it has been our practice to install new lamps at the beginning of each four week period so that illumination values might be easily duplicated. Where lamps of one experiment were carried over to another, yields lower than anticipated were obtained. This obviously should be expected.

10 A Hartford time switch of 220 volt-50 ampere capacity is employed for controlling the daily illumination period (Hartford Time Switch Co., 71 Murray St., N. Y.)

11 It is realized that some light is lost into the surrounding room by this placement of lamps. Theoretically it might be more efficient to have the side walls of the plant chamber of some highly reflecting material and all lamps overhead. The slight loss in light efficiency with our arrangement, granting sufficient intensity for the purpose at hand, is offset by the opportunity given to observe plants and instruments through the glass walls.

12 Gen. Elec. Co. R. L. M. Standard Dome 500.

13 The Gen. Elec. Co. performance curve indicates a 7 per cent. decrease in candle power for this period and for 120 per cent. of the rated life of the lamp, a loss of 20 per cent. HARVEY (3) on one occasion used similar lamps a total of 3000 hours. At this time they were undoubtedly producing less than fifty per cent. of their rated output in foot candles.

Intensity determinations are made at present with a single junction, iron-nickle thermocouple in circuit with a galvanometer sensitive to 10^{-7} amperes. Such an instrument must be used with caution since it measures total energy output rather than that of the visible spectrum. By means of suitable wave filters the instrument can be adapted to determination of energy distribution and by calibration against a standard lamp visible radiation may be recorded in terms of candle power or as ergs per $cm.^2$ per second. The thermocouple is quite sensitive to direction of radiation. It is our practice therefore to place it within a six liter frosted flask, the integration of radiation from different angles thus being made possible. The set up is placed at different positions within the plant chamber and equalization of illumination is easily achieved by adjustment of the lamp standards.[14]

HUMIDITY

Control of humidity is still in the development stage. Although the Bristol system[15] of control has been installed our conditions have not been altogether satisfactory for its successful operation. The apparatus consists of a sensitive nitrogen filled bulb operating in conjunction with a "controller," a relay and a solenoid steam valve. A change in wet bulb temperature as induced by humidity fluctuations, makes or breaks a weak electric circuit controlling the steam valve. Steam injected into the chamber is the humidifying agent. It is planned to construct a large underground chamber as a reservoir of humidified pre-heated air.

The lack of humidity control has not been a serious one in our studies so far. The moisture content of the air at Berkeley is remarkably uniform throughout the year and the fluctuations which have been met have not influenced yields as may be seen by reference to the dry weight column in table II.

[14] The 300 watt Mazda C lamp has a lumen output of 17.7 per watt or a total of 5300 lumens, 12.57 lumens equaling one spherical foot candle. The reflector efficiency is 76 per cent. thus $\frac{5300 \times 76}{100 \times 12.57} = 320.5$ foot candles per lamp. The average of a number of galvanometer readings for a lamp one foot distant was 35 mm., the thermocouple being housed within the frosted flask. With the housed thermocouple in the plant chamber one foot above the perforated platform and at the temperature of the preceding measurement, the galvanometer reading was $\frac{320.5 \times 285}{35} = 2610$ foot candles in the plant chamber. This was the intensity employed for the yields given in table II and is considerably higher than that given for the Boyce Thompson Institute light room where 25000 watts were employed, giving an intensity of 400 foot candles (8). HARVEY (4) lists intensities ranging from 50–10,000 foot candles with the usual range lying between 300–2000 foot candles.

[15] The Bristol Co., Waterbury, Conn.

Cost of equipment

The total cost of the control apparatus including plant chamber and its accessories, light standards, reflectors, fans, temperature and humidity control, but not the thermo-hygrograph, was approximately $500.00 a unit. The plant chambers were constructed in the University shops and the source of the other equipment has been noted in the text. Electric current is the main item of maintenance expense and the amount of this will obviously depend on the nature of the investigation and the local cost of current. For the conditions noted in table II and with current at 3 cents per K.W., the cost per day per unit for lighting, heating and fans was approximately $2.00. The cost of lamps under the same conditions will range from fifty to seventy-five cents per day, depending upon size and frequency of replacement.

Summary

1. Equipment is described which permits the growth of plants under controlled conditions of light, temperature, humidity, and culture solution.

2. The apparatus is constructed in units, thus making possible easy expansion, with enlargement of research program and budget. The initial cost of the unit described was approximately $500, and the cost of maintenance per day of use approximately $2.50, with electricity at three cents per K. W.

3. The data given definitely show the feasibility of establishing an environment which can be duplicated at will, thus leading in turn to the duplication of yields, number of tillers, height of tops, and other external evidences of growth. Such a controlled environment permits the isolation of a single variable and a quantitative study of diverse physiological phenomena.

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16 Since the submission of proof of this manuscript the authors have received a reprint from Prof. H. Lundegårdh of Stockholm on the "Photosynthesis of cultivated plants, its relation to yield and its dependence on climate and soil." (Meddelande N:r 331 från Centralanstalten för försöksväsendet på jordbruksområdet. Avdelningen för lantbruksbotanik N:r 43.) The article is in Swedish but has an excellent summary in English. Lundegårdh describes an apparatus in which plants are grown under controlled light, temperature, humidity, and carbon dioxide. The photographs indicate smaller units than the one herein described. Light is all from above and heat from the lamps is taken care of by a water screen. There seem to be many features about the apparatus that merit attention.

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ALUMINUM TOXICITY[1]

FORMAN T. MCLEAN AND BASIL E. GILBERT

Introduction

This subject has been the occasion for some controversy and misunderstanding among different workers. It has been asserted by a number of workers (**3, 5, 7, 8, 10, 11, 13, 15, 16, 18, 19**) that aluminum salts are decidedly toxic, and that the toxicity of acid soils is due primarily to this cause (**1, 2, 6, 7, 9, 15**). Others maintain that aluminum compounds are so insoluble that they cannot occur in solution in a sufficient concentration to cause injury to plant growth (**4, 12, 13**). Investigations of soils from widely scattered localities indicate that active aluminum is most abundant in leached-out acid soils of humid regions such as Hawaii, the Pacific Northwest, and the Eastern states of the United States.

As was shown in a previous paper (**16**), plants vary in their susceptibility to aluminum poisoning. Lettuce, beets and some other plants are very sensitive, while corn is more resistant and redtop is notably so. The injury caused to sensitive plants is localized mainly in the roots, which are dwarfed and inhibited from branching. The aluminum accumulates in the protoplasm and particularly in the nuclei of the cortex of roots immersed in a nutrient solution containing aluminum. Such accumulation has been noted in plants sensitive to aluminum but not in the roots of resistant plants, even when subjected to high aluminum concentrations. Aluminum poisoning causes the affected plants to absorb dyes, water, and nutrient salts less readily than normal plants.

Aluminum stimulation

Like some other toxic elements, aluminum is stimulating to plants in dilute concentrations. STOKLASA (**21, 22**), MAZÉ (**14**), and others (**20**) have found small quantities to be beneficial.

SERIES I

Cultures were grown in nutrient solutions with distilled water, using the same technique as reported in a previous paper (**16**). The plants were grown in 250-cc. jars, six plants to each jar, and solutions were changed twice weekly, supplying the phosphate and the aluminum at alternate changes. The acidity of the cultures was adjusted to approximately the same pH in all of the cultures in any one series by additions of Na_2CO_3 or

[1] Contribution no. 365 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

of H_2SO_4, so that the cultures containing aluminum were no more acid than the controls. The results are given in table I.

TABLE I

EFFECT OF DILUTE CONCENTRATIONS OF ALUMINUM SALTS ON THE YIELD OF VARIOUS PLANTS GROWN IN DISTILLED WATER SOLUTION CULTURES

AIR-DRY WEIGHTS PER PLANT

CONCENTRATION OF ALUMINUM	Al AS SULPHATE		Al AS CITRATE				
	RYE	OATS	ALFALFA	BUCKWHEAT	OATS	ONIONS	REDTOP
ppm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.0	0.46	1.18	0.190	0.66	1.50	0.17	0.51
3.4	0.86*	....	0.069	0.72	2.26	0.20	0.42
6.8	0.66	1.31	0.264*	0.79*	1.82	0.16	0.22
13.6	0.40	1.23	0.075	0.66	2.58*	0.39*	0.72*
27.2	....	0.86	0.018	0.39	2.36	0.24	0.49

* Optimum growth.

While these results are based on very few plants, six in each culture, and the results are somewhat irregular, the magnitude of the differences are so great that more replications would not be expected to change the main results. They clearly show stimulation by concentrations of 3.4 to 13.6 parts per million of aluminum added to what is ordinarily considered a complete nutrient solution. This table, however, does not include any of the plants which have been found to be markedly sensitive to aluminum poisoning.

Solubility of organic and inorganic aluminum compounds

Most workers have found that inorganic compounds of aluminum, sulphate, and chloride are easily precipitated, and remain in solution only in decidedly acid media, pH 4.5 or less. By alternating the applications of phosphorus salts and aluminum salts at successive changes of the culture solutions, it has been easy to maintain strongly toxic concentrations in solutions held at a pH of 4.0 to 4.5. The solutions containing aluminum were strongly toxic while non-aluminum solutions of approximately the same acidity were not seriously injurious to any of the plants tested.

Aluminum in combination with organic acids was found to be less easily precipitated. In the absence of phosphorus salts in the solution, aluminum citrate remained in solution even in nearly-neutral solutions. This was substantiated by a dialysis experiment, the results of which are given in table II. Twenty-five cc. each of nutrient solutions containing all of the usual salts and in addition 0.24N aluminum citrate, were placed in three dialysis

thimbles impregnated with pyroxylin, and each immersed in 200 cc. of distilled water for seven days. The acidity of the solutions placed inside the thimbles was pH 6.5.

TABLE II

DIFFUSION OF ALUMINUM OUT OF PYROXYLIN THIMBLES INTO DISTILLED WATER AFTER SEVEN DAYS

EXPERIMENTS	AMOUNT OF Al INSIDE THIMBLE		AMOUNT OF Al OUTSIDE THIMBLE	
	gm.	ppm.	gm.	ppm.
Thimble 1	0.0063	250	0.0355	178
Thimble 2	0.0068	271	0.0358	179
Thimble 3	0.0066	263	0.0345	172

Five times as much aluminum diffused out through the pyroxylin membranes as remained behind in the thimbles, thus showing that aluminum citrate diffuses readily at pH 6.5, and is in true solution, not colloidal.

Phosphorus and aluminum

Aluminum is so readily precipitated as aluminum phosphate that this factor has been eliminated in the foregoing experiment (table I), by supplying the plants with aluminum- and phosphorus-containing solutions at alternate changes of the culture solutions. Thus the plants were supplied with aluminum during half of each week and with phosphorus during the other half.

SERIES II

By this procedure the phosphorus and aluminum were supplied separately and the aluminum could not interfere with the absorption of phosphorus by precipitating it from solution outside the plant. However, it has been found that large amounts of phosphate fertilizer supplied to the soil diminished or prevented aluminum toxicity, and it was thought that possibly a larger amount supplied to plants in solution culture might increase their phosphorus content and decrease their susceptibility to aluminum poisoning. This was accordingly tested by supplying plants in 250-cc. glass bottles with two different levels of calcium acid phosphate, alternating with aluminum sulphate. The results are given in table III.

The additional amount of calcium acid phosphate seemed to depress the yields of rye and corn but to affect the yield of lettuce very little. Further, the higher amount of phosphorus did not noticeably reduce the aluminum toxicity except in the case of the highest aluminum dosage in corn and, in this case, the depression in both series of cultures was so severe that the

TABLE III

EFFECT ON ALUMINUM TOXICITY OF DIFFERENT LEVELS OF PHOSPHORUS SUPPLIED TO PLANTS IN SOLUTION

	RYE		CORN			LETTUCE	
	PO_4 SUPPLIED		PO_4 SUPPLIED			PO_4 SUPPLIED	
Al added	0.00096N	0.00288N	0.00096N	0.00288N	Al added	0.0096N	0.00288N
	WEIGHT OF CONTROL PLANTS					WEIGHT OF CONTROL PLANTS	
	gm. 3.46	gm. 1.70	gm. 5.10	gm. 4.04		gm. 5.20	gm. 5.55
	RELATIVE WEIGHT OF PLANTS					RELATIVE WEIGHT OF PLANTS	
ppm.	per cent.	per cent.	per cent.	per cent.	ppm.	per cent.	per cent.
0.0	100	100	100	100	0.0	100	100
3.4	92	92	....	....	1.0	77	54
6.8	69	72	....	....	1.7	90	74
13.6	74	79	96	93	2.3	83	70
27.2	64	65	77	18	3.2	......	79
40.8	64	64	59	51	4.1	80	73
54.4	58	50	12	25	......	......	......

difference between the depression to 12 per cent. and to 25 per cent. yield, respectively, may not be significant. The tap water used in growing these cultures contained approximately 3 to 4 parts per million of aluminum, and was the same for all of the cultures in this series.

SERIES III

To test the effect of aluminum and phosphorus in the nutrient solution together, cultures of barley were started in February, 1927, and grown to maturity in quart Mason jars, 6 plants to each jar, in complete nutrient solutions (**17**), containing both phosphorus and aluminum in the same solution. Further, the solutions were not changed during the course of the experiment, and the cultures had a pH of 4.0 to 4.5 at the beginning, becoming about 5.9 toward the end of the growth period. One-half of the full nutrient supply and one-half of the amounts of aluminum salts intended for each culture were put in at the beginning, and the remainder was supplied at the sixth week, when the plants were half grown. Six plants were placed in each culture, and each culture triplicated with the exception of the last three. One culture not triplicated received extra phosphorus. The yields are summarized in table IV.

The added phosphorus, when it exceeded the equivalent of the added aluminum, proved beneficial when added with the aluminum. This is shown by the yield of culture 15 which exceeded that of the culture receiving no

TABLE IV

YIELDS OF BARLEY FROM SOLUTION CULTURES WITH DIFFERENT AMOUNTS OF PHOSPHORUS AND ALUMINUM (14 AND 27 PPM. RESPECTIVELY)

CULTURE NOS.	1, 2, 3,	4, 5, 6,	10, 11, 12,	13, 14,	15
Treatment per plant	milli-equivalents		milli-equivalents		milli-equivalents
PO_4	0.15	0.15	0.15	0.39	0.63
Al	0.0	0.27	0.54	0.54	0.54
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.
Tops	2.40	1.30	2.00	1.70	2.80
Roots	0.40	0.30	0.25	0.40	0.50

aluminum and with the lower amount of phosphorus. Phosphorus was intentionally made suboptimum in all of this series of cultures.

SERIES IV

Since phosphorus counteracts aluminum toxicity when they are placed in solution together, it would appear that its beneficial action occurred by precipitating the aluminum as aluminum phosphate. But is colloidal aluminum toxic to plants when in contact with the roots? It has been assumed by MAGISTAD (**13**) and others that aluminum is toxic only when in crystalloid form. This was tested by a special arrangement of three cultures grown at the same time as the preceding set. The plants were all grown during the first six weeks in pint jars with nutrient solution like cultures 1, 2, and 3 of the last series. Then their roots were confined in collodion sacks exactly the size of pint jars, and into these sacks were poured the residual solutions from the first six weeks of growth. These sacks containing the plants were suspended in quart jars with the added nutrients for the remainder of the growth period, but omitting the phosphorus. Then to culture 7 the phosphorus was added outside the collodion membrane. Culture 8 received both phosphorus and 1.62 milli-equivalents of aluminum sulphate outside the membrane so that any aluminum or phosphorus would have to pass through the membrane to reach the roots. Culture 9 received the same amounts of aluminum and phosphorus as culture 8, but poured inside the membrane and thus placed within reach of the barley roots which by this time extended to all parts of the space enclosed by the collodion sack.

Culture 9, the roots of which were in contact with the precipitated aluminum, promptly developed typical symptoms of aluminum toxicity, becoming discolored, and ceasing to grow normally. The other two cultures maintained normal roots. The yields of these three cultures are shown in table V.

TABLE V

YIELDS OF BARLEY CULTURES TO SHOW WHETHER COLLOIDAL ALUMINUM IS TOXIC

CULTURAL CONDITIONS	YIELD PER PLANT		P REMAINING	
	ROOTS	TOPS	INSIDE	OUTSIDE
	gm.	gm.	ppm.	ppm.
Culture 7, in collodion sack, nutrients outside, no Al	0.3	1.0	......	......
Culture 8, in collodion sack, nutrients + Al + P outside	0.2	1.6	0.03	0.02
Culture 9, in collodion sack, nutrients outside, Al + P inside	0.2	0.7	0.09	0.13
Culture 5, no collodion sack, nutrients + Al + P	......	......	0.06	......

Thus the aluminum was apparently unable to pass through the collodion membrane, for culture 8 showed no evidence of aluminum toxicity. Culture 9, however, subjected to presumably the same sort of aluminum compounds as were present outside the membrane in culture 8, was severely injured, indicating that colloidal aluminum can still cause toxicity to barley when placed in contact with the roots. If this conclusion is accepted then colloidal aluminum may be toxic to plants growing in soil likewise.

SERIES V

Another series of barley plants was grown to maturity in tall 3-liter biological specimen jars, with more liberal phosphorus supply, to test the effects of added aluminum sulphate and citrate on the yield. Culture solutions were changed and nutrients and aluminum were added weekly. According to the plan developed by PEMBER (17), the amounts of the nutrients were varied with the age of the plants. The cultures were divided into two series; cultures 1 to 12 were to test the toxicity of aluminum sulphate and were maintained quite acid at pH 4.6, while cultures 13 to 24 were to test the toxicity of aluminum citrate. Since aluminum citrate has been found not to precipitate out of solution even when neutralized, these latter cultures were maintained at pH 6.0. The even numbered cultures 14 to 24 inclusive, were grown with distilled water, while the others were grown with well water. The nutrient solution contained calcium nitrate, potassium chloride, magnesium sulphate and ferric nitrate. The results are shown in table VI.

Aluminum, either sulphate or citrate, was toxic with the addition of 16 parts per million, and about equally so either with the lesser or greater amount of phosphorus. In the distilled water cultures, there being no alu-

TABLE VI

YIELDS OF BARLEY IN SOLUTION CULTURES SUPPLIED WEEKLY WITH VARYING AMOUNTS OF NUTRIENTS, BUT WITH UNIFORM AMOUNTS OF ALUMINUM AS SULPHATE OR CITRATE (5 AND 16 PPM. RESPECTIVELY)

ALUMINUM SULPHATE

CULTURE NOS.	1, 2	3, 4	5, 6	7, 8	9, 10	11, 12	
Treatment per plant	milli-equivalents			milli-equivalents			
PO_4	0.216	0.216	0.216	0.648	0.648	0.648	Alternating
Al	0.0	0.201	0.603	0.0	0.201	0.603	
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.	gm.	
Straw	1.8	1.2	0.5	1.3	1.7	0.7	
Grain	0.5	0.5	0.1	0.6	0.6	0.1	

ALUMINUM CITRATE

CULTURE NOS.	14	16	18	20	22	24	
Treatment per plant	milli-equivalents			milli-equivalents			
PO_4	0.216	0.216	0.216	0.648	0.648	0.648	Alternating
Al	0.0	0.201	0.603	0.0	0.603	0.300	
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.	gm.	
Straw	1.3	1.1	0.8	1.3	0.9	1.7	
Grain	0.6	0.3	0.1	0.5	0.2	0.7	

CULTURES WITH DISTILLED WATER

CULTURE NOS.	13	15	17	19	21	23	
Treatment per plant	milli-equivalents			milli-equivalents			
PO_4	0.216	0.216	0.216	0.648	0.648	0.648	Alternating
Al	0.0	0.201	0.603	0.0	0.603	0.300	
Air-dry weight per plant	gm.	gm.	gm.	gm.	gm.	gm.	
Straw	0.80	0.8	0.80	0.4	1.2	1.0	
Grain	0.05	0.3	0.03	0.1	0.2	0.1	

minum in the basal solution, 5 parts per million of aluminum was more beneficial than none at all, again showing aluminum stimulation; and 16 parts per million was evidently deleterious to the grain yield.

Summary

A number of different kinds of plants was grown in solution cultures to study their susceptibility to injury by aluminum and to test the efficiency

of the phosphate radical as a preventive of aluminum injury. Very low concentrations of aluminum, 3 to 13 parts per million, were found to be stimulating to plants, while higher concentrations were toxic. Aluminum in combination with organic acids, such as citric and tartaric, was found to be dialyzable and to be toxic at very low acidities, pH 6.5 or even less acid. So high acidity is not an invariable or necessary accompaniment of aluminum toxicity. Further, non-dialyzing forms of aluminum which could not pass through a collodion membrane in sufficient amounts to cause toxicity, were decidedly toxic when placed in contact with the roots of barley.

Phosphorus, when put into solution with aluminum compounds, completely overcame the toxicity of the aluminum when the concentration of the phosphorus in the form of phosphate was equivalent to that of the aluminum. This was presumably brought about by the precipitation of the aluminum as aluminum phosphate, as additional amounts of phosphate, in excess of an amount needed for nutrition, exerted no beneficial effect whatever when it was supplied alternately with the aluminum at successive changes of the culture solution, so that no precipitation of aluminum phosphate could take place in the solution outside of the plants.

Conclusions

1. Soluble phosphate, in concentrations equivalent to that of aluminum, completely counteracts aluminum toxicity.

2. Aluminum, even when in the medium in non-diffusible, colloidal form can induce toxicity when in contact with roots of barley.

3. Aluminum as citrate is toxic even in solutions with very low acidity, pH 6 +.

4. Aluminum is stimulating to plants at low concentrations, and toxic at higher ones.

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SOME STUDIES ON THE HARDINESS OF CERTAIN SPECIES OF *VACCINIUM*[1]

W. G. Brierley and A. C. Hildreth[2]

For the past twelve years the Minnesota Agricultural Experiment Station has been studying the possibilities of bringing the blueberry (*Vaccinium pennsylvanicum* Lam., *V. canadense* Kalm., and *V. corymbosum* L.) under field culture. The work has been hampered at times by winter injury to the plants. Such injury has occurred nearly every year with *V. corymbosum,* the common high bush blueberry of the east, and has been met with more or less frequently in the two native species. This recurring injury has led to a study of the hardiness of these species.

Observations in the field have shown that the most severe injury in the case of *V. corymbosum* has occurred in late fall. Under conditions prevailing at the Forest Experiment Station, Cloquet, where the field work is carried on, this species usually grows late, failing to mature its wood before the advent of severe freezing weather. Usually early snows have served to protect the lower portions of the new shoots, but the upper portions exposed to temperatures no lower than $-10°$ C. generally have been killed. Injury of this sort probably is due to lack of maturity and consequent lack of hardiness, rather than to inability to endure such temperatures in seasons which permit normal maturity and hardening off.

It appears likely that the lack of maturity in the shoots of *V. corymbosum* at Cloquet may be due in part to a short growing season. Blossoming and the beginning of shoot growth in this and the native species does not occur until late in May. Frosts in May or early June frequently are the cause of light crops through injury to the blossoms. Frosts at this time of year also are usually accompanied by low daily maximum temperatures under which shoot growth takes place slowly if at all. Light frosts frequently occur in late August and killing frosts are common in early September. Under such fall conditions shoots of *V. corymbosum* are frequently exposed to killing frosts before the formation of winter buds at the tips of the shoots has progressed very far, and before the normal maturing of the foliage has occurred. This is the condition of shoots described as poorly matured or showing a lack of maturity. Full maturity in the shoots of *V.*

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[2] The authors wish to express their appreciation of the assistance and cooperation in these studies of Prof. T. S. Hanson, Assistant Superintendent, Forest Experiment Station, Cloquet, Minn.

corymbosum as evidenced by well-developed buds at the tips and by maturity of foliage has occurred only once (1926) during the twelve seasons this species has been grown at Cloquet.

The native low bush species *V. pennsylvanicum* and *V. canadense* occur over a more northerly range than the *V. corymbosum.* It was thought, therefore, that they might be greatly superior in hardiness to the latter and could be used as a source of hardy parents in the production of hybrids with the *V. corymbosum,* which, because of its height and bush-form is a more desirable horticultural type. However, in the spring of 1924 extensive winter injury was noted in these native species, both in the cultivated plots and in the wild. In this season there was no snow cover until mid-January and sub-zero (F.) weather had been frequent. Again in the spring of 1925 similar injury was noted. This winter also was characterized by low temperatures in December with little snow. The question was raised therefore, whether the native species are materially hardier than *V. corymbosum* or whether they merely escape injury most years by reason of their low growth habit which enables them to be protected by a minimum snow cover.

Studies to determine the relative cold resistance of matured shoots of the three species were begun in the fall of 1925. Controlled temperatures under artificial refrigeration were employed, the freezing apparatus and methods being the same as used by HILDRETH (3) in determining the relative hardiness of apple varieties. Samples of new shoots and older wood were collected at Cloquet on October 22 after exposure in the field to a minimum temperature of $-10.5°$ C. At this time the new shoots of *V. corymbosum* were showing the characteristic injury resulting from freezing while immature. A few exceptionally vigorous shoots of *V. pennsylvanicum* also showed this type of injury although not to the same extent as *V. corymbosum.* Through a misunderstanding this material was subjected to a minimum temperature of $-12°$ C. in the freezing chamber, only 1.5° C. lower than the minimum to which they had previously been exposed in the field. After standing for some time with the butts in water in a cool greenhouse the samples showed no injury except as observed in the field.

Shoots of *V. pennsylvanicum* and *V. corymbosum* collected at Cloquet on January 26, 1926, were placed in a cool greenhouse with the butts in water. As the buds pushed into growth it was evident that there was only a little more injury at the tips than was found in the samples collected in October. It should be noted that although the air temperature had fallen as low as $-32°$ C. in December, the plants of *V. pennsylvanicum* were protected by snow and were probably not exposed to such a low temperature. The snow did not entirely cover the plants of *V. corymbosum,* but the exposed tips had already been killed in October as previously mentioned. Evidently the

snow had been sufficient to protect these plants against serious additional injury.

The value of snow protection for blueberries has been pointed out by COVILLE (2). He notes that in February, 1918, shoots of low bush hybrids were killed at Whitesbog, New Jersey, at a temperature of about $-12°$ F. while at Greenfield, New Hampshire, the parent stock, when protected by snow was uninjured although here the temperature dropped to $-30°$ F. The same season at Crotched Mountain, N. H., he found that tops of high bush blueberries, which projected above the snow, were killed back while the bases and sides of the same bushes which had been protected by snow bore the usual crop of berries.

Summarizing the results for the winter of 1925–1926, it appears that killing in the three species studied was due largely to a combination of immaturity plus early cold. The most severe injury occurred in October at temperatures no lower than $-10.5°$ C. *V. corymbosum*, which probably was not well matured, suffered most. Winter cold caused very little additional injury as the plants presumably were protected by snow.

In the fall of 1926 material was again taken for freezing tests, collection being made at Cloquet, October 21. Most of the new shoots were matured to the tip buds. Only a few of the very vigorous and immature shoots of *V. corymbosum* and *V. pennsylvanicum* showed slight freezing at the tips. Temperature records at Cloquet during the months of September and October showed a gradual decline, a minimum of $-6°$ C. having been experienced up to the time the material was collected.

Such fall weather conditions are generally considered to favor the development of hardiness. WINKLER (5) has shown that a gradually declining temperature increases the cold resistance in woody plants. REIN (4) and others have found that northern plants are able to accommodate themselves to cold, the plants becoming more resistant by exposure to temperatures that are relatively low but still above the lethal point. Under the conditions which prevailed at Cloquet this fall, it is assumed that full maturity was reached and that these species had attained about their maximum hardiness for the time of year.

After collection the samples were wrapped in damp moss and kept cool to hold them in condition till freezing tests could be made. On October 23 one lot of shoots from each species was exposed for three hours to a temperature of $-16°$ C. A second lot was similarly exposed on the following day to $-20°$ C. and a third lot on the 25th to $-24.5°$ C. All lots after freezing were stored in damp moss until October 26th when they were placed with the butts in water in a cool greenhouse. Three weeks later the shoots were examined and compared with control samples to determine the extent of

injury from the three treatments. The results obtained are shown in the accompanying table I.

TABLE I

THE EFFECTS OF LOW TEMPERATURES ON MATURE SHOOTS OF THREE SPECIES OF *Vaccinium*
ALL LOTS EXPOSED FOR THREE HOURS IN A FREEZING CHAMBER

TEMPERATURE TREATMENT	*V. pennsylvanicum*	*V. canadense*	*V. corymbosum*
– 16° C.	Alive to tip buds All buds starting	Alive to tip buds Buds starting slowly	Alive to tips[1] Buds starting slowly Wood showing injury
– 20° C.	Alive to tip buds All buds starting	Buds not starting Wood color dull	Buds not starting Wood injury intensified
– 24.5° C.	Buds starting slowly Wood showing slight injury	Buds not starting Wood discolored	Buds not starting Wood discolored

[1] Except in the case of shoots killed at tips by freezing in the field.

It will be seen in this table that at – 16° C., *V. pennsylvanicum* was uninjured. In the other two species injury at this temperature was indicated by a slower pushing of buds. In *V. corymbosum* there was also a slight discoloration of the wood.

At – 20° C., *V. pennsylvanicum* still appeared uninjured with practically all buds starting to grow. *V. canadense* showed no buds starting and the normal greenish color of the wood was noticeably dulled. *V. corymbosum* was badly injured or killed with no buds starting and the wood had a water-soaked appearance.

At – 24.5° C., *V. pennsylvanicum* showed considerable injury with the buds starting slowly and the wood somewhat discolored. The other two species showed no sign of bud activity and the wood was darkened and had a water-soaked appearance. In *V. canadense* at this temperature the phloem was noticeably browned. Apparently this temperature was almost low enough to kill *V. pennsylvanicum* and did kill the other species.

As a further check on killing temperatures 15 to 20 shoots of *V. pennsylvanicum* and *V. canadense* were dug from beneath the snow at Cloquet on February 19, 1927, and frozen on February 28 for three hours at – 32° C. All of these shoots were killed except two shoots of *V. pennsylvanicum* which started a very few buds in a feeble manner after four weeks in the greenhouse.

These data show that the killing points of *V. corymbosum* and *V. canadense* at the end of October lie somewhere between – 16° and – 20° C., with *V. canadense* only slightly hardier than *V. corymbosum.* As *V. pennsylvanicum* was injured at – 24.5° in October and killed at – 32° in February,

it appears that the killing point of this species in October lies not far below $-24.5°$ C. It is also evident that this species did not increase in hardiness to any appreciable extent after October.

In conclusion, it appears from the results of freezing tests that the killing points of all three species as grown in Minnesota lie fairly close together. This fact indicates that there is no great difference in the actual hardiness of the three species. Of still greater significance is the apparent inability of any of them to endure very low temperatures. Comparing the killing points of these *Vaccinium* species with those of orchard and forest trees which grow in northern climates (**1**, **2**, **5**) it is seen that the former develop relatively little cold resistance. Even the hardiest species, *V. pennsylvanicum,* apparently cannot endure exposure to such winter temperatures as commonly prevail in the regions to which it is native. As *V. pennsylvanicum* and *V. canadense* occur naturally over a more northerly and westerly range than *V. corymbosum,* in a region of low winter temperature but ordinarily with ample snow cover, it is safe to conclude that these two species are protected rather than inherently hardy. The adaptation of these species to cold climates appears to be largely an ecological one rather than any physiological adjustment of the tissues to extremely low temperatures. In the matter of avoiding cold injury in the fall, *V. pennsylvanicum* seems to have a slight advantage over the other two species, since it attains greater hardiness early in the fall when a few degrees of cold resistance may be sufficient to prevent killing back of the shoots. This fall hardiness, however, is probably only another result of the dwarf habit, the shorter terminal growths maturing earlier and becoming more hardened before severe freezing weather occurs.

With this relative lack of hardiness in all three species, it would seem futile to attempt to develop from this material tall hybrids which would be likely to grow late or to extend above the snow cover. If a truly cold resistant blueberry is to be developed apparently it will be necessary to search outside of these three species for hardy parent stocks.

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CERTAIN PHYSICOCHEMICAL PROPERTIES OF PINEAPPLE STEM COLLOIDS*

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(WITH EIGHT FIGURES)

The investigations reported in this paper were conducted with the purpose of obtaining certain information on the behavior of pineapple plant tissue colloids in health and disease. Some of this information is reported in another publication by the writer (**7**).

It was found (**6**) about two years ago that pineapple plants grown in water and soil cultures of different H-ion concentrations thrive best at pH values between 4.5 and 6.5. These findinge suggested that the inhibitory effect of H-ions above or below the values mentioned may have had some influence on the physicochemical properties of the pineapple tissue colloids. As such studies could not have been conducted very satisfactorily with the living tissues, it was thought best to obtain the fluid colloids and study them *in vitro*.

Methods of experimentation

Pineapple stems, from which the leaves, the extreme basal portion, and part of the exterior tissues of the base had been removed, were ground in a meat grinder. The pulpy mass was then placed in a fruit press and the fluids extracted by pressure. The extract was filtered through filter-paper for the removal of particles of plant tissue. The product thus obtained was centrifuged for further purification by means of a Sharples centrifuge, and then subjected to the treatments, as outlined later, for the separation of the different colloidal fractions. The reagents used for these treatments were in certain cases 0.1 normal NaOH and HCl, in others 0.1 normal NaOH and HNO_3, and 0.1 normal ammonium hydroxide and acetic acid. The pH of the resulting solution never went above 10.0 nor below 2.0.

The procedure followed for the treatment of the different colloid fractions may be illustrated by the following outline and diagram.

OUTLINE

1. Extracted pineapple fluids.
2. Treatment of pineapple fluids with NH_4OH. Formation of a precipitate (P–2) and filtrate (F–2).

* Technical paper no. 4 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

3. Treatment of precipitate (P–2) with HNO_3. Formation of a precipitate (P–3) and filtrate (F–3).
4. Treatment of filtrate (F–2) with acetic acid. Formation of precipitate (P–4) and filtrate (F–4).
5. Treatment of filtrate (F–3) with NaOH. Formation of precipitate (P–5) and filtrate (F–5).
6. Treatment of filtrate (F–4) with NaOH. Formation of a precipitate (P–6) and filtrate (F–6).
7. Treatment of filtrate (F–5) with HNO_3. Formation of a precipitate (P–7) and a filtrate (F–7).
8. Treatment of filtrate (F–6) with HNO_3. Formation of a precipitate (P–8) and filtrate (F–8).

DIAGRAM

- Pineapple fluids
  - Fluids + NH_4OH
    - Precipitate (P–2) + HNO_3
      - Precipitate (P–3)
      - Filtrate (F–3) + NaOH
        - Precipitate (P–5)
        - Filtrate (F–5) + HNO_3
          - Precipitate (P–7)
          - Filtrate (F–7)
    - Filtrate (F–2) + acetic acid
      - Precipitate (P–4)
      - Filtrate (F–4) + NaOH
        - Precipitate (P–6)
        - Filtrate (F–6) + HNO_3
          - Precipitate (P–8)
          - Filtrate (F–8)

Qualitative chemical tests of different fractions

The different fractions were analyzed qualitatively with the following results:

TABLE I

QUALITATIVE TESTS ON THE STEM COLLOIDS

Tests	Different colloid fractions					
	P–3	P–4	P–5	P–6	P–7	P–8
Biuret	+++	+++	—	—	+	+
Sulphur	—	—	—	—	+	+
Xanthoproteic	+	+	—	—	+	+
Molisch	—	—	+	+	—	—

The six different fractions may be placed in three groups as the result of their chemical behavior. P–3 and P–4 represent one and the same protein; P–7 and P–8 likewise, represent another protein, and P–5 and P–6 represent a carbohydrate.

Physicochemical properties of the different fractions

The different fractions were purified as thoroughly as possible by dialysis through parchment paper. Folded parchment paper cones containing the different colloidal fractions were immersed in four-liter beakers of distilled water. The water was changed three times a day. A piece of thymol, about the size of a pea, was kept in the water to prevent biological action. The electrical resistance of the dialysate was determined every

TABLE II

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-3 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION 24 HOURS		TURBIDITY	$AgNO_3$	$K_4Fe(CN)_6$	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH	AFTER TREATMENT	AFTER STERILIZATION				
	cc.	cc.	pH	pH				
1	5.0		2.25	2.30	–	–	–	Soluble
2	4.4		2.40	2.60	–	–	–	"
3	3.8		2.50	2.65	–	–	–	"
4	3.2		2.70	2.85	+	–	+++	Insoluble
5	2.6		2.80	3.20	++	–	++	"
6	2.0		3.15	4.10	+++	–	++	"
7	1.4		4.10	4.65	++++	–	+	"
8	0.8		5.00	4.85	++++	–	–	Insoluble (isoelectric point)
9	0.45		5.80	4.85	++++	–	–	Insoluble
10	0.22		6.20	4.85	++++	+	–	"
11	0	0	6.47	5.10	++++	+	–	"
12		0.3	6.65	5.50	+++	++	–	"
13		0.7	6.70	5.60	+++	++	–	"
14		1.3	6.90	6.05	++	+++	–	"
15		1.9	7.15	6.20	++	+++	–	"
16		2.5	7.30	6.60	+	+++	–	"
17		3.1	7.50	7.00	–	–	–	Soluble
18		3.7	7.80	7.50	–	–	–	"
19		4.3	8.10	7.80	–	–	–	"
20		4.9	8.60	8.00	–	–	–	"

time the solution was changed. After this value had risen from 200 to 15,000 ohms, the nitrate content from the added HNO_3 of the dialysate, was also determined. It was found that when an electrical resistance of about 20,000 ohms had been reached, the nitrate content of the dialysate was considerably less than one part per million. At this stage the different fractions were removed from the bags and studied for their physicochemical behavior.

Each fraction was made up to a certain volume with distilled water. This was acted upon, then, by a mechanical stirrer, in order to disperse evenly through the solution, the different sizes of the colloidal particles,

TABLE III

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-4 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION	TURBIDITY	$AgNO_3$	$K_4Fe(CN)_6$	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH					
	cc.	cc.	pH				
1	5.0		2.10	–	–	–	Soluble
2	4.5		2.15	–	–	–	"
3	4.0		2.20	–	–	–	"
4	3.5		2.25	–	–	–	"
5	3.0		2.35	–	–	–	"
6	2.5		2.40	–	–	–	"
7	2.0		2.45	–	–	–	"
8	1.5		2.80	–	–	–	"
9	1.0		3.20	+	–	+++	Insoluble
10	0.5		3.80	++	–	+++	"
11	0.25		4.40	+++	–	+++	"
12	0.125		4.60	+++	–	++	"
Check	0	0	4.65	++++	–	++	"
14		0.125	4.90	+++++	–	+	Insoluble (isoelectric point)
15		0.25	4.95	+++++	–	–	Insoluble
16		0.5	5.10	++++	–	–	"
17		1.0	5.30	+++	+	–	"
18		1.5	5.40	++	+	–	"
19		2.0	5.75	++	++	–	"
20		2.5	6.00	+	++	–	"
21		3.0	6.15	+	+++	–	"
22		3.5	6.35	+	+++	–	"
23		4.0	6.50	–	–	–	Soluble
24		4.5	6.65	–	–	–	"
25		5.0	6.75	–	–	–	"

before the solution was distributed into a number of Erlenmeyer flasks. Each flask received 100 cc. of the colloidal suspension drawn off by means of a pipette. The different flasks received, in addition to the colloidal suspension, a definite volume of 0.1 normal HNO_3 or NaOH. The number of cubic centimeters of the acid or alkali solution together with the pH value and turbidity that were produced are reported in tables II, III, IV, V and VI, and in fig. 7. In tables II and III and fig. 8, the reactivity of the different solutions with such salts as $K_4Fe(CN)_6$ and $AgNO_3$ also are recorded. The method adopted for determining the reactivity of these salts with protein P–3 and P–4 was as follows: Protein solutions, about 25 cc. of each, were taken into a dark room and there treated with 5 cc. of 1 per cent. concentration of either salt. The mixture was left to stand over night and was then filtered through hardened filter-paper. The residue was collected and placed in a solution having the same pH as that of the original solution. The intensity of the color of the precipitate served as an index of its reactivity. It may be added that before removing the precipitate from the filter-paper it is necessary to wash it with distilled water repeatedly until all traces of the unaffected salt have been removed. Equally good results were obtained when protein suspensions of different

TABLE IV

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-7 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		REACTION	TURBIDITY	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH			
	cc.	cc.	pH		
1	4.0		2.63	–	Soluble
2	3.5		2.67	–	ʻʻ
3	3.0		2.70	–	ʻʻ
4	2.5		2.88	–	ʻʻ
5	2.0		3.00	+	Insoluble
6	1.5		3.13	+	ʻʻ
7	1.0		3.33	++	ʻʻ
8	0.5		4.16	+++	ʻʻ
9	0.25		5.32	+++	ʻʻ
10	0.125		6.45	++++	Insoluble (isoelectric point)
11	0	0	7.30	+++	ʻʻ
12		0.125	8.65	++	ʻʻ
13		0.25	9.45	+	ʻʻ
14		0.5	9.90	–	Soluble
15		1.0	10.42	–	ʻʻ
16		1.5	10.58	–	ʻʻ
17		2.0	11.00	–	ʻʻ

hydrogen-ion concentrations were treated with the above salts and left in darkness over night without subjecting the resulting solution to filtration. The intensity of the color that develops in the solutions after they have been exposed to the light may serve, in this case also, as an index of the reactivity of the salts and protein.

TABLE V

PHYSICOCHEMICAL BEHAVIOR OF FRACTION P-8 IN ACIDS AND ALKALIES

SAMPLE NUMBER	REAGENT		pH	TURBIDITY	REMARKS
	0.1 N. HNO_3	0.1 N. NaOH			
	cc.	cc.			
1	5.0		2.00	–	Soluble
2	4.5		2.10	–	"
3	4.0		2.35	–	"
4	3.5		2.45	–	"
5	3.0		2.55	+	Insoluble
6	2.5		2.75	+	"
7	2.0		3.10	++	"
8	1.5		3.50	++	"
9	1.0		3.90	+++	"
10	0.5		5.35	+++	"
11	0.25		6.65	++++	Insoluble (isoelectric point)
12	0.125		7.20	+++	"
13	0.	0.	7.50	++	"
14		0.125	7.95	++	"
15		0.25	8.75	+	"
16		0.5	9.82	–	Soluble
17		1.0	10.37	–	"
18		1.5	10.85	–	"
19		2.0	11.00	–	"

Discussion of results

The results presented in tables II, III, IV, V and VI indicate that the difference in the physicochemical behavior of protein colloids and carbohydrate colloids is quite pronounced. The former react amphoterically and have a definite isoelectric point, whereas the latter do not. Different proteins may have different isoelectric points as is the case with fractions P-3 and P-4, on the one hand, and P-7 and P-8 on the other.

The amphoteric behavior of the two proteins is due, according to LOEB (3) and others, to their component reactive groups, namely, the amino (NH_2) and carboxyl (COOH) radicals, the former behaving as a cation, and the latter as an anion. In the case of the carbohydrate colloids, where

usually only one reactive group occurs, that is, the carboxyl radical, the behavior can not be amphoteric but points only in one direction. The experimental data are in complete agreement, therefore, with the theoretical expectations.

TABLE VI

BEHAVIOR OF SAMPLES P-5 AND P-6 IN ACIDS AND ALKALIES

Sample number	Reagent 0.1 N. HNO_3	Reagent 0.1 N. NaOH	Reaction	Turbidity
	cc.	cc.	pH	
1	12.0	10.0	2.30	++++
2	8.0		2.50	+
3	6.0		2.90	+
4	4.0		5.30	++
5	2.0		6.70	++
6	1.0		7.2	+++
7	0.5		7.3	+++
8	0.0	0.0	7.4	++++
9		0.5	7.6	++++
10		1.0	7.7	++++
11		2.0	7.9	++++
12		4.0	8.4	++++
13		6.0	8.5	++++
14		8.0	8.6	++++
15		10.0	8.8	++++

During the different studies both the protein and carbohydrate colloids were subjected to various treatments. One hundred cc. of suspensions of protein P-3 and P-5 of pH values above and below the isoelectric point were treated with 10 cc. of 0.1 molal NaOH and HCl. The water of the suspension was evaporated at room temperature and the residue containing NaCl crystals and protein was examined under the microscope. The examination proved that at pH values above the isoelectric point of the protein the protein occupied part of the central portion of the crystal, whereas the arrangement was reversed at pH values below the isoelectric point, as shown by figs. 1, 2 and 3. At the isoelectric point the protein forms transparent thread-like structures, which, under the microscope, are transparent flakes without any definite arrangement.

When the carbohydrate colloids, of fractions P-5 and P-6, dissolved in a beaker or other container, were treated at intervals with drops of 0.1 normal NaOH, it was found that certain membranous spherical or ring bodies are formed, as shown in fig. 4. These bodies are hollow inside; that

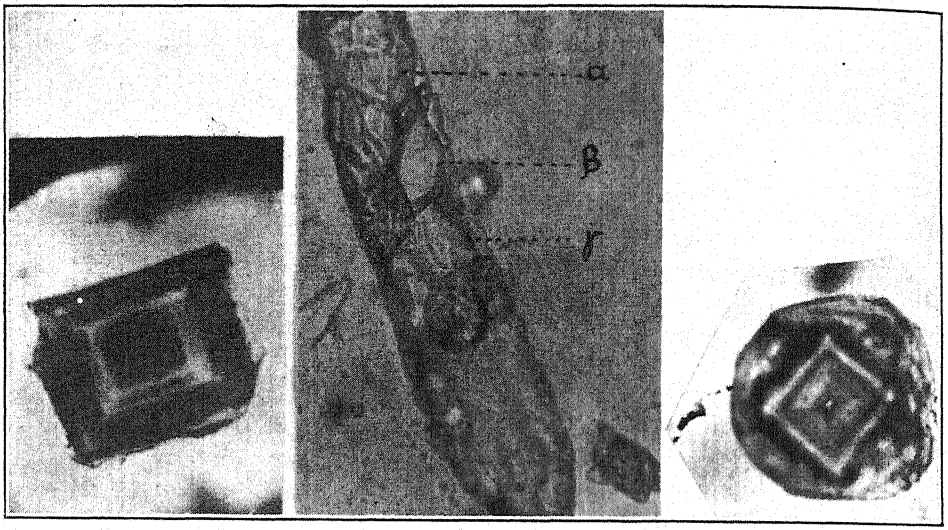

FIG. 1. Sodium chloride crystals in pineapple protein (P-7) suspension, at pH values above 6.5.

FIG. 2. Isoelectric pineapple protein (P-7) in an imperfect crystalline (α, β, and γ) form.

FIG. 3. Sodium chloride crystals in pineapple protein (P-7) suspension at pH values below 6.3.

is, the solid substance that enters into their composition does not fill all the space but like a rubber balloon forms a membranous shell. The thickness of the membrane of such bodies may vary from 0.5 to 0.05 mm. They may remain in the solution from a few seconds to many minutes depending on the acidity or alkalinity of the solution. If the solution is too acid their duration is short; if, however, it is but slightly acid or close to neutral they may last for many minutes and possibly hours. Their formation may be explained as follows: The COOH radical of this colloid fraction being chemically highly reactive is able to enter into a chemical combination with substances having an opposite electric charge and to form salts which may or may not be soluble. In this particular case the COOH reacting with NaOH according to the equation,—$RCOOH + NaOH \rightarrow RCOONa + H_2O$,—forms a certain insoluble salt. Whether the reaction, as stated, represents the actual conditions is not known. Qualitative tests of the carbohydrate colloid gave a positive test for calcium. The presence of calcium suggests that the substance may be a calcium pectate or some other similar salt. Such a salt when acted upon by an acid or a base may behave as follows:

(1) $(RCOO)_2Ca + 2HNO_3 \rightarrow 2RCOOH + Ca(NO_3)_2$.

(2) $2RCOOH + Ca(NO_3)_2 + 2NaOH \rightarrow (RCOO)_2Ca + 2NaNO_3 + 2H_2O$.

These reactions represent the conditions that might possibly develop with the different treatments. The equations are merely illustrative, and the balancing should not be interpreted as indicative of precise knowledge of the reactions.

The spherical or doughnut shaped bodies that are formed, when this carbohydrate colloid is treated with NaOH, may be the insoluble salt of calcium pectate or some other similar substance. The physicochemical conditions that enter into their formation may be explained as follows: The COOH radical upon coming in contact with the NaOH of the solution forms water while at the same time the H-ion is replaced by Na or Ca. With the replacement of the H-ion by Ca the membrane of the body or calcium pectate salt is formed. The water that is being formed is apparently sur-

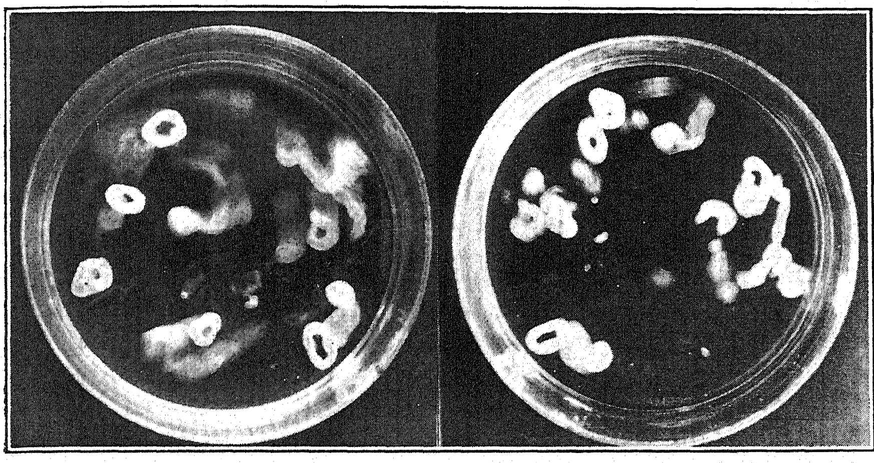

FIG. 4. Carbohydrate colloid bodies of spherical and ring shapes formed by adding drops of 0.1 N. or 1.0 N. NaOH in an acid suspension of the colloids. The bodies are membranous, containing in their cavities water and some salts.

rounded by the membrane with the Na or Ca ions projecting into it. The inert part of the colloid substance or R occupying the exterior surface of the membrane projects into the exterior acid solution. With conditions like these it is possible to have the membrane lasting in the acid solution from a few minutes to a few hours. The decomposition or dissolution of the membrane may be brought about by the interplay of osmotic forces. The H-ions that are present in the outside solution penetrate the membrane either slowly or rapidly depending on their concentration. Their penetration into the interior of the spherical body may reverse the reaction, that is, the H-ion may replace the Ca or Na ions and reestablish the original condition as follows: $(COO)_2Ca + 2HNO_3 \rightarrow 2COOH + Ca(NO_3)_2$.

According to these observations, then, the structure of a carbohydrate colloid body, such as the one under discussion, may be imagined as a hollow sphere the shell of which is formed by aggregated micellae held in close proximity by electrostatic forces as represented by the sketch in fig. 5. Membranous bodies of this type have been observed to form in different sizes, varying between 10 and 0.5 mm.

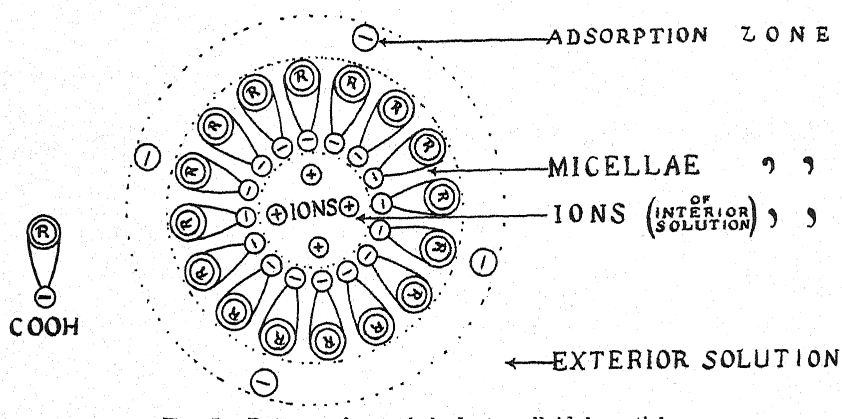

FIG. 5. Pattern of a carbohydrate colloidal particle.

The question now arises: Is it possible to have similar bodies formed with proteins at pH values above or below their isoelectric points? Assuming that isoelectric protein is like a collapsed rubber balloon or a flat membrane, is it not possible for such a membrane to assume a spherical or other similar form when suspended in solutions of a higher or lower pH from that of the isoelectric point of the protein? By analogy, one would expect in the case of the formation of spherical bodies, the COOH group to project inward at pH values above the isoelectric point and outward at pH values below this point. The position of the NH_2 group must be then, exactly the reverse of that of the COOH group, as in fig. 6. Although the work of previous investigators favors this view considerably, no one has ever been able to demonstrate that such a structure occurs in proteins. MEYER (4) has observed with starch and water the formation of a net structure made up distinctly of globules. HARDY (2) believes that concentrated gelatin jellies consist of drops of water suspended in a gelatin-rich phase. LOEB's (3) work on the swelling of proteins, suggests that proteins must undergo a certain modification in their isoelectric structure to make them hold water very tenaciously. From a theoretical consideration the formation of such membranous structures is possible only at pH values slightly above or below the isoelectric point of the particular protein. At considerably higher concentrations of H^+ or OH^- ions the electrostatic forces that keep the indi-

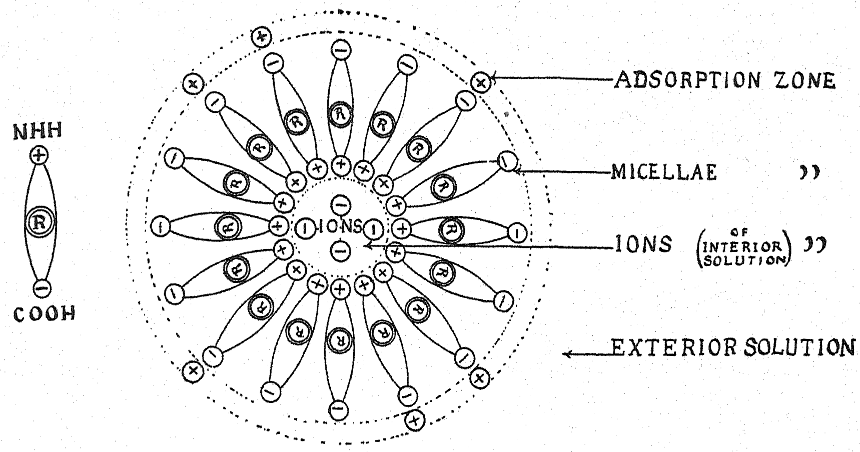

FIG. 6. Pattern of a protein particle.

vidual micellae together in a membrane-like structure are destroyed. The development of this condition is due to the increase of the electrical potential of the micellae. These, acted upon by high concentrations of H^+ or OH^-

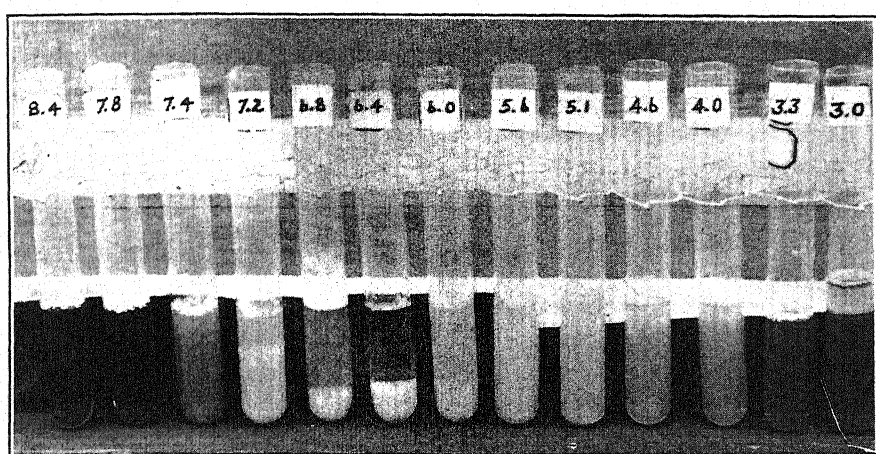

FIG. 7. Pineapple protein (P-7) in solution of different pH values. At 6.4, the isoelectric point, the protein precipitated completely; slightly at pH 6.8, 6.0, and 7.2; remained turbid at 5.6, 5.1, 4.6, 4.0, and 7.4, but dissolved completely at 7.8, 8.4, 3.3 and 3.0.

ions, become highly electrified, which condition makes them repel each other and disperse themselves thoroughly in the solution.

The phenomenon of dispersion and precipitation has been studied quite extensively by LOEB (**3**), NORTHROP (**5**), FAURÉ-FREMIET and NICHITA (**1**)

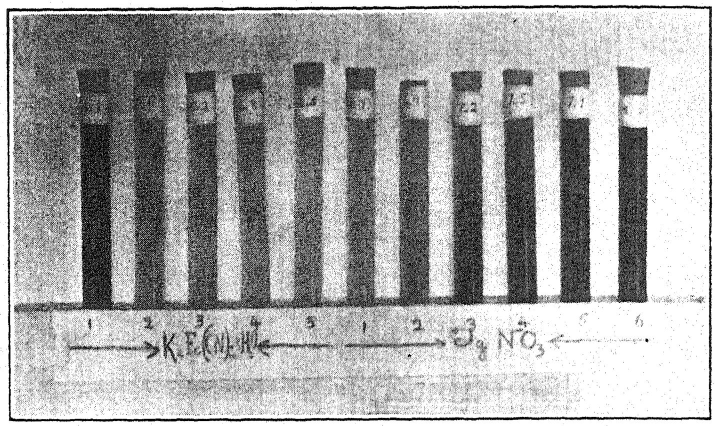

FIG. 8. Pineapple protein (P-7) treated with $K_4Fe(CN)_6$ and $AgNO_3$. It reacted well, and developed a yellowish green color at pH 5.6 and 6.2, which did not photograph well; at 3.1 it was green. With $AgNO_3$ it reacted well, as shown at pH 6.7–8.5.

and others. These investigators found that the stability of colloidal particles or bacteria in a suspension depends on the electrical potential of the solution. When this potential reaches a very low figure the particles or bacteria, as the case may be, become precipitated.

Further work on the structure of protein as well as carbohydrate colloids is necessary in order to obtain a clearer picture of their physical and chemical behavior.

Summary

The results obtained by the investigations reported in this paper indicate that it is possible to separate mixtures of colloids by making use of their isoelectric or critical precipitation point. Both carbohydrate and protein colloids may be separated in this way.

The writer expresses an opinion about the structure of colloidal particles which may be represented by a membranous body of spherical or other shape, the interior space of which contains water, with the reacting radical of the colloid and other ions in solution.

Pineapple stem protein has been obtained in an imperfectly crystalline form. The crystalline structure is flake-like, transparent, and macroscopically fibrous. The isoelectric point of this protein is at pH 6.4.

The writer wishes to express his appreciation to Dr. A. L. DEAN for reading the manuscript, for his constructive criticisms, and many helpful suggestions.

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THE QUANTITATIVE DETERMINATION OF CHLOROPHYLL[1]

F. M. Schertz

(WITH TWO FIGURES)

Introduction

Up to the present time many methods have been used to determine the amount of chlorophyll present in a plant or in a given solution. Not one of the methods has been generally adopted by those who desire to estimate chlorophyll quantitatively. Each worker apparently has adopted a method of his own. It is clearly evident then that attempts at standardization should be made. The methods as given here are an attempt to place the estimation of chlorophyll on a sound basis, so that all workers may be able to compare their data. The results reported are not to be considered as final but only a report of progress in the development of better methods of quantitative research in this field.

Methods which have been used to estimate chlorophyll

No attempt will be made to refer to all of the methods which have been used for this kind of work but rather a brief description will be given of a few of the more important contributions.

Perhaps the first attempt at measuring the concentration of chlorophyll was made by Monteverde (**7**) when he photographed the spectra of several different concentrations of the alcoholic extracts of green leaves. His purpose was not to discover a method for quantitative work but rather to ascertain the nature of chlorophyll spectroscopically.

In 1910 extinction coefficient values were used by Malarski and Marchlewski (**6**) to measure the concentration of colored solutions. Chlorophyll was converted into chlorophyllan (pheophytin) and the absorption of this solution was measured by means of the König-Martens spectrophotometer.

Jacobson and Marchlewski (**3**) later used the photographic method in their study of the chlorophyllans from various leaves. By this method a direct comparison of the amount of chlorophyllan present could be made, for several photographs could be taken on the same plate. The method was used to show that chlorophyll consisted of two components, allo- and neochlorophyll. The procedure was then modified by Jacobson (**2**) in order to determine the amount of the two chlorophyllans present in 1 gram or less of leaf.

[1] Soil-Fertility Investigations, U. S. Department of Agriculture, Washington, D. C.

Later MONTEVERDE and LUBIMENKO (8) used a spectrocolorimeter, which was specially constructed for their use by Leitz, in determining the amount of chlorophyll, carotin and xanthophyll present in the leaves of different plants.

WEIGERT (10) recognized the practical importance of knowledge regarding the spectrophotometric curves of the four chloroplast pigments. His figures show the extinction curves that are formed by solutions of the leaf pigments in pure acetone. He also explains how quantitative results may be obtained from such curves. No practical application, however, has ever been made of his results.

Considerable work has been done by HENRICI (1) on the chlorophyll content of alpine and lowland plants. Her methods were based upon those used by WILLSTÄTTER. The chlorophyll standard for the colorimetric determinations was an alcoholic solution of crude chlorophyll made from fresh nettle leaves. No attempt was made to place the amount of chlorophyll present in the leaves upon an absolute basis.

Total chlorophyll and total carotinoids were estimated in the red algae by WURMSER and DUCLAUX (12). They made no attempt to separate the two chlorophylls or to separate carotin from xanthophyll. The chlorophylls as such were determined spectrophotometrically by measuring the absorption at wave-length 670 mμ, and the carotinoids were determined by using wave-length 450 mμ. The amount of pigment present in the red varieties was expressed as 100 while the determinations for the green varieties were based upon the red and the results are given in per cent. The separation of the pigment was a modification of the procedure as given by WILLSTÄTTER and STOLL.

MAIWALD (5), by using methods based upon WILLSTÄTTER and STOLL's procedure, has determined the amount of chlorophyll present in potato leaves. A mixture of pure chlorophyll ($\alpha + \beta$) (obtained from WILLSTÄTTER) was used as a standard and comparisons were made by using a Duboscq colorimeter.

By means of the spectrocolorimeter, LUBIMENKO (4) has quantitatively investigated the amount of chlorophyll present in marine algae. He used crystallized chlorophyll as a basis for his comparisons. The instrument is represented as being quite accurate, though no figures are given to show its degree of accuracy.

It will be observed that most of the methods used are only a modification of the procedure described by WILLSTÄTTER and STOLL (11). In their methods WILLSTÄTTER and STOLL separated chlorophyll into its two components, α and β, by converting them into their respective derivatives, phytochlorin *e* and phytorhodin *g*. The other workers have all determined only the total amount of chlorophyll which was present in the material.

Procedure

In this paper only the data for estimating total chlorophyll will be given, because the estimation of chlorophyll α and chlorophyll β by the methods of WILLSTÄTTER and STOLL have proved quite unsatisfactory. The manner of preparing a chlorophyll extract from fresh green leaves for use in such quantitative work has been presented (**9**). Pure chlorophyll $(\alpha+\beta)$ has been used as a basis for the curves obtained by means of the Duboscq colorimeter and also for those obtained by means of the Hilger wave-length spectrometer. In either case 0.0507[2] gm. of pure chlorophyll $(\alpha+\beta)$ was dissolved in 50 cc. of ether. The chlorophyll in the ether was then saponified by shaking the solution for 15 min. or more with 10 cc. of cold concentrated methyl alcoholic potash. The ether was removed by evaporation, using reduced pressure. Distilled water was added to make a volume of 250 cc. Dilutions as required were made from this solution of potassium chlorophyllin $(\alpha+\beta)$, and the pure chlorophyll was measured as potassium chlorophyllin.

The colorimetric method

The solution as described above was diluted and readings were made, using a combination of 3, 4, and 5 blue plus 10 and 20 yellow Lovibond slides as a standard. The chlorophyllin solution was matched against the Lovibond slides by comparing the depth of tint and not by attempting to match exactly the color of the slides, which would be impossible.

The combination of Lovibond slides used here does not exactly match the color of the chlorophyll solution. The writer used this combination to aid in determining the purity of his samples, for some fixed standard was absolutely necessary since no known pure chlorophyll was at hand. No combination of Lovibond slides will match all of the samples of chlorophyll, for the proportions of α and β vary. The tint of the chlorophyll solutions will vary accordingly. In quantitative work on fresh green leaves the color variations in the green pigments being tested will be even greater than in the case of pure chlorophyll. The ideal method, of course, is to use pure chlorophyll as a standard; but this will not be found to be practicable because pure chlorophyll is not available commercially, and moreover it is not advisable for each worker to prepare his own pure product. A company in America is now attempting to prepare pure chlorophyll and it is hoped that chlorophyll of known purity soon may be purchasable.

The results of colorimetric readings for six different samples are given in table I. In the case of samples 3, 4, 5, and 6, more than one weighing

[2] 0.0507 gm. was used, for when 0.0500 gm. of chlorophyll $(\alpha+\beta)$ was dried at 100° C. it lost an average of 0.0007 gm.

TABLE I

COLORIMETRIC READINGS OF AQUEOUS SOLUTIONS OF POTASSIUM CHLOROPHYLLIN $(\alpha + \beta)$ MEASURED AGAINST A COMBINATION OF LOVIBOND SLIDES

SAMPLE NO.	GRAMS PER LITER			
	0.20	0.15	0.10	0.05
	mm.	mm.	mm.	mm.
1	8.7	12.3	17.7	33.5[a]
2	8.5	11.6	16.1	35.0
3	9.1	10.6	17.4	33.5
3	8.5	....	16.3	30.2
4	8.4	....	16.8	32.1
4	9.0	12.2	17.8	31.9
5	7.5	....	15.0	29.8
5	7.6	9.5	14.2	29.3
6	9.5	....	17.8	32.8
6	7.7	9.6	15.7	28.6
6	9.6	12.8	19.6	34.9
Average	8.55	11.22	16.76	31.96

[a] Each number is an average of 3 readings on the colorimeter measured against the following combination of Lovibond slides, 3, 4 and 5 blue plus 10 and 20 yellow. Measurements were made on different days as well as different times of the day so as to get an average result.

was made and another set of readings was taken on a different day. This was done to obtain as good an average as possible, for it is almost impossible to duplicate exactly any set of readings on a given sample. Using the average of the figures for each concentration in table I, a curve has been

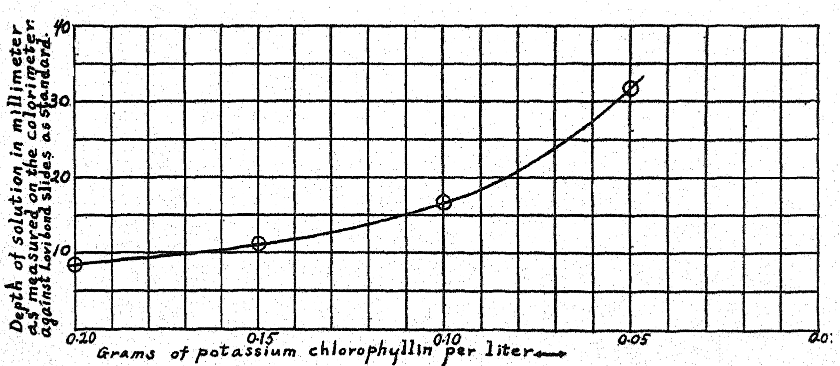

FIG. 1. Colorimetric determination of chlorophyll.

drawn, fig. 1, with grams of chlorophyll $(\alpha + \beta)$ per liter represented on the x-axis and depth of solution in millimeters as measured on a colorimeter

represented on the y-axis. The curve may be used by those desiring to know approximately the amount of chlorophyll present in a given solution. However, it is advisable to use pure chlorophyll as a standard if it is obtainable.

In order to know the accuracy of the method the maximum error has been calculated for each of the four concentrations. The high, the low and the average reading together with their respective values in terms of chlorophyll are given in table II. Calculating, using the values for grams of

TABLE II

DATA, OBTAINED FROM TABLE I, USED IN CALCULATING THE PROBABLE ERROR OF A SINGLE DETERMINATION

	CONCENTRATION IN GRAMS PER LITER			
	0.20	0.15	0.10	0.05
	mm.	mm.	mm.	mm.
Highest reading	9.6	12.8	19.6	35.0
Lowest reading	7.5	9.5	14.2	28.6
Average reading	8.55	11.22	16.7	31.9

From the curve in fig. 1, these readings are interpreted in terms of grams of chlorophyll per liter respectively as follows:

0.173	0.133	0.083	0.047
0.235	0.175	0.120	0.054
0.200	0.150	0.100	0.050

chlorophyll per liter in table II, it is found that the maximum difference in the readings for concentration 0.20 is $0.235–0.173 = 0.062$, and $0.062 = 31$ per cent.; for 0.15 it is 28 per cent., for 0.10, 37 per cent., and for 0.05, 14 per cent.

From table I, the probable error of a single observation has been calculated. The calculations are rather lengthy and need not be given here, since the method of calculating is shown later in connection with the spectrometric method. For readings made with the concentrations 0.20, 0.15, 0.10 and 0.05, the respective probable errors in per cent. are $\pm$ 7.4, $\pm$ 7.3, $\pm$ 7.0 and $\pm$ 3.3.

The spectrometric method

The same solutions and concentrations used in the colorimetric method were used here also. The slit opening was set at 5, and a 200-watt electric light was used as the source of illumination. The widths of the absorption band (I) are given in table III. Only one set of readings was taken in

TABLE III

POSITION OF THE EDGES OF THE ABSORPTION BAND (I) OF PURE POTASSIUM CHLOROPHYLLIN ($\alpha + \beta$) (THICKNESS OF SOLUTION = 10 MM.)

SAMPLE	CONCENTRATION (GRAMS PER LITER)			
	0.20	0.15	0.10	0.05
1	668–605* 667–607 666–607	660–615 659–615 659–615	656–619 654–619 655–619	647–624 646–624 646–624
2	666–609 666–609 665–609	663–611 662–612 661–612	656–617 656–617 656–618	646–625 647–623 648–624
3	665–607 666–604 667–605	....	656–614 656–616 655–616	650–621 649–621 650–622
4	665–607 664–608 663–608	....	656–617 654–619 654–617	651–624 648–621 649–624
5	671–605 670–605 669–607	664–613 662–612 662–612	656–617 656–618 657–618	648–624 646–624 646–624
6	670–604 670–604 668–606	664–611 662–611 662–612	657–617 657–617 657–616	648–623 647–623 648–624
Average	667–606.4	661.7–612.6	655.7–617.3	647.7–623.3

* All readings are reported in mμ.

the case of each sample of chlorophyll ($\alpha + \beta$). From the average results in table III the graph in fig. 2 has been constructed. From this graph readings from a solution which contains an unknown amount of potassium chlorophyllin, may be interpreted in grams of chlorophyll per liter.

In order to find the maximum error in the spectrometric determinations in table III, the widths of the absorption band have been interpreted in grams of chlorophyll per liter and are recorded in table IV. From table IV the maximum difference in the determinations may be calculated as follows: For concentration 0.20 grams per liter it is 0.2340–0.182 = 0.0520 and $\frac{0.0520}{0.2055} = 25$ per cent.; for concentration 0.15 it is found to be 18.9 per cent., for concentration 0.10, 19.4 per cent., for 0.05, 53.2 per cent.

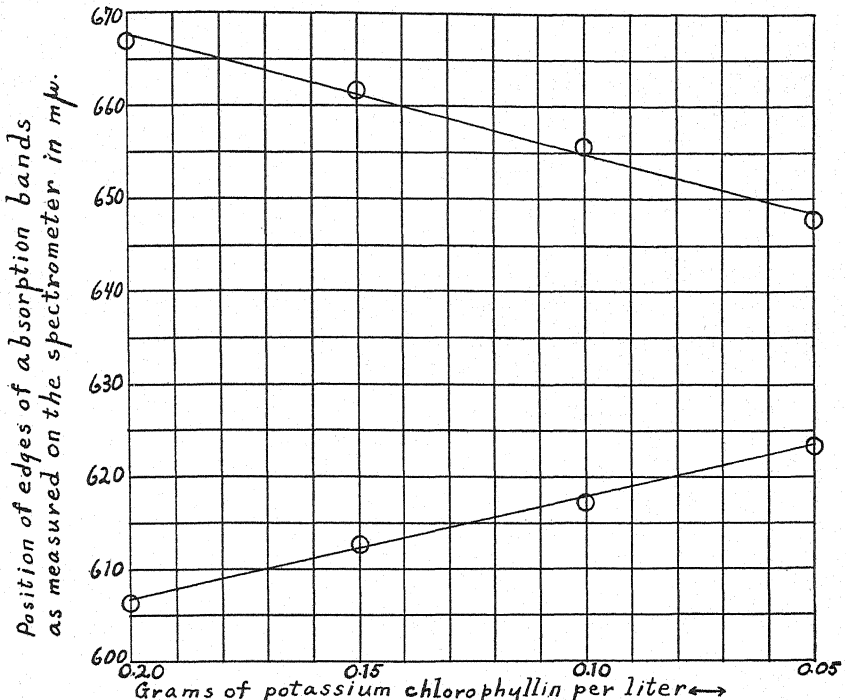

FIG. 2. Spectrometric determination of chlorophyll.

TABLE IV

DATA, OBTAINED FROM TABLE III, WHICH WAS USED IN CALCULATING THE ERROR OF A SINGLE DETERMINATION

SAMPLE	mμ (x)	mμ (x)	mμ (x)	mμ (x)
1	$60\frac{2}{3}$ = 0.200	$44\frac{1}{3}$ = 0.133	36 = 0.098	$22\frac{1}{3}$ = 0.040
2	$56\frac{2}{3}$ = 0.183	$51\frac{2}{3}$ = 0.159	$36\frac{2}{3}$ = 0.107	23 = 0.042
3	$60\frac{2}{3}$ = 0.200	....	41 = 0.119	$28\frac{1}{3}$ = 0.065
4	$56\frac{1}{3}$ = 0.182	....	37 = 0.102	$26\frac{1}{3}$ = 0.057
5	$64\frac{1}{3}$ = 0.233	$50\frac{1}{3}$ = 0.158	$38\frac{2}{3}$ = 0.107	$22\frac{2}{3}$ = 0.041
6	$64\frac{2}{3}$ = 0.234	$51\frac{1}{3}$ = 0.162	$40\frac{1}{3}$ = 0.116	$24\frac{1}{3}$ = 0.048
Lowest reading	0.1820	0.1330	0.0980	0.0400
Highest reading	0.2340	0.1620	0.1190	0.0650
Average reading	0.2055	0.1530	0.1081	0.0480

(x) = grams of chlorophyll per liter.
mμ = width of absorption band in mμ.

The probable error of a single determination has also been calculated from table III. The calculations will not be given but the results may be obtained by using the equation:

$$r = 0.6745\sqrt{\frac{\Sigma V^2}{N-1}}$$

in which r is the probable error, 0.6745 is a constant, N is the number of determinations and V is the variation of each reading from the average reading.

The probable error for a concentration of 0.20 gm. per liter is ± 7.5 per cent., for 0.15 it is ± 5.8 per cent., for 0.10 it is ± 5.0 per cent., and for 0.05 it is ± 14.0 per cent.

It will perhaps be best to give an example to explain how the spectrometric graph is used. Using a given solution of potassium chlorophyllin the absorption band is found to extend from 620–652 mμ; then the width of the band is 32 mμ. In fig. 2 a place is found where the distance between the lines is 32 mμ. By inspection of the figure it is seen that this corresponds to 0.08 gm. of chlorophyll. So the solution, the absorption bands of which have been measured in the spectrometer, contains 0.08 gm. of chlorophyll per liter.

In table V a comparison is made of the results obtained by using the

TABLE V

COMPARISON OF THE ACCURACY OF THE COLORIMETER AND THE SPECTROMETER

ERRORS OF DETERMINATION	CONCENTRATION OF POTASSIUM CHLOROPHYLLIN IN GRAMS PER LITER			
	0.20	0.15	0.10	0.05
		Colorimeter results		
Maximum error	31 per cent.	28 per cent.	37 per cent.	14 per cent.
Probable error	± 7.4	± 7.3	± 7.0	± 3.3
		Spectrometer results		
Maximum error	24.0 per cent.	18.9 per cent.	19.4 per cent.	53.2 per cent.
Probable error	± 7.5	± 5.8	± 5.0	± 14.0

colorimeter with the results obtained on the spectrometer. It is seen that the results on the colorimeter are best when the concentration is about 0.05 gm. per liter while the results on the spectrometer are best with a concentration of 0.10 to 0.15 gm. per liter.

In the absence of better methods for the quantitative determination of chlorophyll either method should give results which are quite satisfactory. Methods have been used here in which it is not necessary to use pure chlorophyll as a standard because of the difficulty of obtaining this pigment pure.

The preparation of pure chlorophyll would be quite a task for many workers who are interested in knowing something regarding the pigment content of plants which are being investigated. Some of the difficulties in preparing pure chlorophyll will be taken up at a later time. The methods as outlined should at least aid in any preliminary investigation of chlorophyll. Of course, the ideal standard is pure chlorophyll; but until the pure pigment is obtainable commercially workers will have to be contented with other methods.

Stability of potassium chlorophyllin solutions

In connection with methods for estimating chlorophyll, investigators should know something regarding the stability of chlorophyllin solutions. Solutions of chlorophyll which had been saponified with methyl alcoholic potash were kept for ten days and the resulting decomposition is shown in table VI. The combination of Lovibond slides described under the colorimetric method was used here to obtain the colorimetric readings.

TABLE VI

KEEPING QUALITIES OF POTASSIUM CHLOROPHYLLIN, STORED AT ROOM TEMPERATURE IN DARKNESS, COLORIMETRIC METHOD

Date	Number of days	Sample			
		1	2	3	4
		mm.	mm.	mm.	mm.
January 17	0	24.9	27.5	25.6	24.8
January 20	3	26.3	32.9	31.2	29.3
January 23	6	42.3	47.4	38.2	32.2
January 27	10	49.0	61.0	49.0	41.0

TABLE VII

KEEPING QUALITIES OF POTASSIUM CHLOROPHYLLIN STORED IN THE ICE BOX IN DARKNESS

Date	Number of days	Reading in mm.
June 10	0	10.3
June 12	2	10.3
June 26	16	11.0
July 22	42	11.4
August 14	65	11.7

However, a solution which had been kept in the ice box showed very little decomposition (table VII) even when stored for 65 days. The safer

practice would be to estimate the chlorophyll within a day or two after it had been prepared from the leaf material, meanwhile keeping the solutions stored in the ice box.

Potassium chlorophyllin is one of the most easily prepared of the chlorophyll products and is much more stable than any of the chlorophyll solutions, consequently it has been used as a basis for the determination of chlorophyll. Also, in separating chlorophyll from the yellow accompanying pigments it is necessary to saponify the chlorophyll to chlorophyllin. If any other derivative of chlorophyll were used in the estimation of chlorophyll our methods of separating the pigments would have to be modified considerably. Consequently, the methods as offered in this paper have been based upon the use of potassium chlorophyllin.

Discussion

This paper is concerned primarily with the methods now available for determining the amount of chlorophyll. It seems desirable in conclusion to say something about the future possibilities of determining chlorophyll. The methods as given here are not very accurate, being only good enough for preliminary work. Many problems concerning the estimation of chlorophyll demand an accuracy as great as has been obtained in determining carotin and xanthophyll. Data showing the complete spectrophotometric curves for carotin and xanthophyll have been obtained at the Bureau of Standards and it is hoped that this data will soon be published.

Before much more progress can be made with chlorophyll it will be necessary to have a complete spectrophotometric curve of chlorophyll α and chlorophyll β. This can be accomplished only after much more is known about chlorophyll than we know at the present time. Practically nothing is known at present about how to prepare a solution of chlorophyll so that its spectral transmission properties can be measured before the chlorophyll is altered by the solvent or by the light used in the study.

Before satisfactory solutions of chlorophyll α or β can be prepared, pigments of undoubted purity must be obtained. The preparation of such pure pigments demands most painstaking chemical technique. Thus far only one chemist has succeeded in preparing solutions of the permitted food dyes pure enough for satisfactory spectrophotometric analysis. It is not to be expected, then, that the preparation of pure chlorophyll α and β will be easily accomplished. Satisfactory spectrophotometric curves for the chlorophyll pigments will be obtained only with great effort and at considerable cost.

Difficult as the problem is, it can be solved if only the determination to solve it is present, but the solution will not be an easy one. While such

work has its practical value, the first consideration should be to gain more information about a substance so prominent everywhere in nature. Methods for the absolutely accurate quantitative determination of the green plant pigments will make it possible to know much more about the rôle of chlorophyll in everyday life. It is hoped that many plant physiologists will become interested in the nature and properties of this pigment which is known to play so important a rôle in plant life. It may be possible that a study of the effect of different wave-lengths of light upon the molecule of chlorophyll in pure solution will reveal much concerning the real nature of light effects upon plant growth and the nature of chlorophyll itself. The work will be most difficult, but since methods are being developed for investigations of this kind, sustained efforts should be made toward a detailed knowledge of the rôle of chlorophyll. The possibility for new discoveries is great.

This paper is only a report of progress in the efforts made to estimate chlorophyll with accuracy, efforts which have not yet met with entire success. More means should be available for purely scientific studies of the nature and functions of the α and β chlorophylls. At present we will have to be contented to work with the tools we have until more and better investigators become seriously interested in chlorophyll problems. Before such serious interest may be developed in the field of chlorophyll chemistry, it may be necessary to develop new points of view of the possible functions of chlorophyll, and the broad significance it may have in the whole realm of plant and animal life.

Summary

1. A brief description is given of the more important methods which have been used to determine chlorophyll quantitatively.

2. A method for determining chlorophyll colorimetrically is described and a graph is given from which quantitative data may be computed.

3. A spectrometric method of determining chlorophyll is described and a graph has been constructed from which chlorophyll may be quantitatively determined.

4. The colorimetric method is more accurate at concentrations of about 0.05 gm. per liter while the spectrometric method is more accurate at concentrations of 0.10 to 0.15 gm. per liter.

5. Chlorophyll solutions which have been saponified with methyl alcoholic potash should not be allowed to stand for any length of time before their pigment content is estimated, since the saponified chlorophyll is rather unstable.

6. The spectrophotometric method offers great promise of being a very accurate method for determining chlorophyll, though the data for the method are yet to be obtained.

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BIOCHEMICAL STUDIES ON SEED VIABILITY
I. MEASUREMENTS OF CONDUCTANCE AND REDUCTION

R. P. Hibbard and E. V. Miller

(WITH TWO FIGURES)

Introduction

Previous work (9) on this problem led to the conclusion that a correlation existed between electrical conductivity of seed extracts and seed viability. With the broadening out of the work and an improvement in the method it was hoped that this conclusion would be still further substantiated. The studies here reported therefore deal with further investigations on this and related lines.

Such work, based on permeability, must necessarily be far from complete in its elucidation, since we have as yet no precise knowledge of the phenomenon itself. That permeability exists in seeds is self-evident but no one has yet offered a plausible explanation. It has been known for some time that a definite relation exists between the permeability of the cell membrane and its injury (19), and it might readily be inferred that a variation in degree of permeability would be correlated with similar variations in degree of injury. The degree of permeability may be indicated by measuring the resistance of the solution in which the seeds have been immersed. Many tests have shown that dead seeds are more permeable than viable ones, so that one might naturally expect that a variation in permeability would be correlated with variations in germination. Thus, a series showing variations in germination in increments of two or three per cent., on the range of zero to one hundred, might be worked out. Obviously, this would be more desirable than merely to know that a seed is dead or is viable.

Historical

Conductance methods

The use of conductance methods for measurements in physiological research dates back to the work of Edward Weber (26) in 1836 and Du Bois-Reymond (8) in 1849. Ranke (21) in 1865 noticed the decrease in resistance in plant and animal tissues upon death.

Brooks (2) in 1923 worked with *Laminaria*, yeast, bacteria, and blood cells. He showed that during the progress of heating of *Laminaria* the "net conductance" approached a constant value which he considered indicative of death. With *Bacillus coli*, his results are rather variable.

JOHNSON and GREEN (**12**) have shown that upon death the conductivity of yeast cells increases, this being due both to exosmosis of salts and to decrease in size of cells.

The outstanding investigator in the field of electrical conductivity measurements of permeability in plant cells is OSTERHOUT (**18**), who recommends this method and shows that the results do not vary more than one per cent. from the mean. In a later work (**19**) he pointed out that the time curve expressing the increase in permeability of *Nitella* during the progress of dying is practically the same whether derived from measurements of exomosis or electrical resistance.

OTHER METHODS

The problem of viability of seeds has been attacked from several different points of vantage. DARSIE and ELLIOTT (**7**), working with PEIRCE in 1914 noticed that the heat of respiration was greater for live seeds than for dead ones. Heat measurements were made under adiabatic conditions, the seeds being placed in silvered Dewar flasks under suitable conditions for germination. These authors claimed that there was a "normal temperature" for each species of plant and that departure from this temperature indicated departures from the best conditions of the organism. Excess of normal temperature indicates infection, while subnormal temperature is indicative of lessened vigor, usually due to increased age. The authors did not make any great claims as to the accuracy of this method for determining viability, but it is evident that seeds of high, low, and medium viability only could be thus determined.

LESAGE (**14**) evolved a method in which seeds were soaked in solutions of KOH of strengths varying from normal to N/682. Non-germinating seeds imparted a color to all solutions, while the viable seeds colored the strong solutions and those down to N/32, but had no noticeable effect upon weaker solutions.

Many workers have attacked the problem of seed viability from the standpoint of enzyme activity. KASTLE (**13**) in his classical work on oxidases, states that peroxidases and catalases are even more widely distributed in living tissues than are oxidases.

Peroxidases attack hydrogen peroxide and liberate atomic oxygen. Their presence may be demonstrated by the fact that they cause bluing of guaiacum. Samples are ground, a drop or two of guaiacum added, and two to three cubic centimeters of neutral hydrogen peroxide introduced. If the peroxidases are present, the guaiacum becomes blue, the intensity of color being proportional to the degree of enzyme action.

McHargue (**16**) has applied this peroxidase test to seeds of corn, hemp, tomato, oat, cow pea, soy bean, castor bean, and lettuce. He claims that these seeds, when exhibiting zero germination, displayed no peroxidase reaction. He goes still further and claims that the peroxidase reaction might be used for seed testing and that seeds of high, low, and medium viability might be thus classified.

On the other hand, Brocq-Rosseu and Gain (**1**) studied the peroxidase activity of seeds ranging from two years to five thousand years old. They found peroxidase activity exhibited in a wheat sample 2000 years old and claim that wheat will retain peroxidase activity 100 years after it loses its ability to germinate.

The enzyme catalase has also been employed for determining viability of seeds. This enzyme attacks hydrogen peroxide and liberates molecular oxygen. A known weight of the powdered sample is mixed with a known volume of neutral hydrogen peroxide and the volume of oxygen evolved is measured. Crocker and Harrington (**6**) determined the catalase activity of Johnson grass and Sudan grass seeds, of the same sample, before and after they had germinated. It was found that the catalase activity increased with germination, thus paralleling respiratory intensity. With *Amaranthus* seeds no correlation was found to exist between catalase and respiratory intensity, vitality, and age. They conclude that generally there is a close correlation between catalase activity and respiratory intensity, but not a very close correlation between either of these and the vitality of the seed or the vigor of the seedlings.

Němec and Duchon (**17**) demonstrated a close correlation between catalase activity and viability of seeds, being able to obtain a difference between seeds varying not more than two or three per cent. in germination. These workers employed cereal grains, legumes, and other seeds.

In 1924 Marotta and Kaminka (**15**) found that Němec's catalase method could not be applied to wheat seeds. Shull and Davis (**24**) have found a relationship between catalase activity and delayed germination in *Xanthium* seeds. There is a decrease in catalase activity in delayed germination. In an earlier work with *Xanthium* seeds, Shull (**23**) showed that oxygen accelerates germination and that a greater amount of oxygen is absorbed with the seed coats removed. Crocker (**5**) showed that oxygen increases respiration and in this way initiates germination.

Since catalase is an oxidizing enzyme and believed to participate in some manner in respiration, it may be this connection with vital activities that suggested the large amount of work attempting to correlate catalase with viability.

All of the evidence herein quoted seems to furnish direct confirmation of the conception of enzymatic activity and its relation to vital phenomena

as held by PALLADIN (20, p. 173) who says: "Life processes are not to be interpreted simply as enzymatic activity." This writer continues to show how organisms might be killed without destroying the enzymes and that enzyme activity is always exhibited by freshly killed tissues where precaution was not previously taken to destroy the enzymes, and that the only difference between enzyme activity in living tissues and dead ones is that in the former they are organized in their work.

We have thus attempted to review briefly the work that has been done toward shortening the method of determining viability of seeds. The majority of the workers quoted here have sought to correlate enzymatic activity with viability. Though enzymes are originally associated with living organisms, we have attempted to show in this historical review that they are not necessarily to be taken as indices of life in organisms.

Catalase and peroxidases might be classified as respiratory enzymes and for this reason may be thought to be concerned with vital phenomena. On the other hand, both dead and living seeds respire and respiratory measurements would be of little value for determining viability. Again any carbon compound might be oxidized to carbon dioxide and this process thus be taken for one of respiration.

The deeper we penetrate into this problem the more complex it grows. It practically resolves itself into the question of "When is a seed dead?" or it becomes a matter of evolving a physico-chemical means of determining the difference between life and death. Has this been determined even for the higher organisms? We are told that death is gradual even in man.

Whether there be a chemico-physical difference between life and death, it seems that there should be a correlation between viability and permeability of plant cells. It seems reasonable to suppose that non-living cells should be more permeable than living ones and that the salts should leach out more rapidly from the dead cells than from the live ones. The amount of salts diffusing out of the cells of the seeds should then modify the resistance of distilled water in which the seeds were soaking and this change of resistance could thus be measured electrically. An attempt was made to measure this change in resistance and to correlate it with viability of seeds. Suitable apparatus was assembled for making the determination.

Apparatus and method

With the exception of a few minor changes the apparatus recommended in the paper by HIBBARD and CHAPMAN (10) was used. The immersion type of electrolytic cell, the best for this kind of work, was thrust into the liquid in which the seeds were soaking.

A stirring apparatus was set up so that possible error due to hand agitation would be avoided. A weighed amount of seeds was poured into

each of the four clean pyrex beakers containing 100 cc. of liquid. The beakers were then placed in a constant temperature water bath at 25° C. A friction-drive universal motor was connected to the four stirrers so that the four samples should be run simultaneously. The electrodes which were immersed in the beakers were connected to a four-way switch for greater convenience.

The most radical departure from the method of FICK and HIBBARD (9) was made in the substitution of dilute $KMnO_4$ solutions for the conductivity water in the measurements of resistance. A number of references have been made above to the work on enzymatic activity as related to viability. KASTLE (13) mentions the fact that catalases, which occur in plant extracts and which liberate molecular oxygen from hydrogen peroxide, will reduce other oxidizing agents, among them being $KMnO_4$. While making some preliminary tests for catalase it was noticed that seeds reduced very dilute solutions of $KMnO_4$ at different rates.

The thought occurred that along with electrolytes there were substances of organic nature leaching out of the seed and hence not measurable by conductivity methods as previously used. This being true, the distilled water might be replaced by the $KMnO_4$ solution and the change in resistance noted as the permanganate is reduced.

Electrical resistance of solutions containing seeds of varying viability

EXPERIMENT I

Beakers containing 100 cc. of M/20,000 $KMnO_4$ were placed in the water bath, the electrodes inserted and connected, and the stirrers started. The seed samples were then added to each beaker, permitting one minute to elapse between the addition of subsequent samples, in order that the resistance readings might be made in the same order. The quantity of seeds used was one gram of timothy, or 50 peas, or 100 wheat seeds. Resistances were determined and the readings recorded at regular intervals. The results are presented in tables I, II and III. For the sake of brevity the figures which represent the resistance of the original $KMnO_4$ solution plus that of the salts and organic substances leached out of the seeds will hereafter be designated as "solution resistance."

The wheat seeds were obtained from the Farm Crops Department of Michigan State College. The variety was Red Rock and the samples varied in age. One hundred seeds were taken from each sample, after all broken, discolored, and infected seeds had been discarded. These were germinated and the percentage germination found was taken as that of the sample. In the above table four different samples having three different germination percentages were taken for the "solution resistance" tests.

TABLE I

SOLUTION RESISTANCE OF WHEAT IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

SAMPLE	GERMINATION	SOLUTION RESISTANCE	AVERAGE
	per cent.	ohms	ohms
108	86	7361	7361
101	86	7364	7364
100	81	7241	7292
100	81	7361	
102	22	4663	5119
102	22	5576	

These garden pea samples were obtained from the Michigan Farm Bureau. The variety was Alaska. Those selected for this experiment varied in germination percentages, as may be seen from the table, from 97 to 63 representing samples of varying ages. History of these samples are not known, but the variety was good, and germination was especially high for the most recently harvested ones. The chief idea here as in the

TABLE II

SOLUTION RESISTANCE OF PEAS IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

SAMPLE	GERMINATION	SOLUTION RESISTANCE	AVERAGE
	per cent.	ohms	ohms
110	97	12880	12985
110	97	13090	
106	93	16250	15945
106	93	15640	
107	93	16320	16145
107	93	15970	
111	92	16320	13355
111	92	16390	
112	90	11600	10800
112	90	10000	
109	83	9048	9729
109	83	10410	
103	76	10660	10535
103	76	8051	
105	75	11830	10072
105	75	8315	
104	63	5699	5518
104	63	5337	

other seed samples where the samples were not described in detail was to get good samples of different ages and different germination percentages.

TABLE III

SOLUTION RESISTANCE OF TIMOTHY IN SAMPLES OF DIFFERENT GERMINATION PERCENTAGES

SAMPLE	GERMINATION	SOLUTION RESISTANCE	AVERAGE
	per cent.	ohms	ohms
58	81	6340	6340
68	70	4970	4795
68	70	4620	
57	54	5974	5715
57	54	5456	
65	0	4205	4266
65	0	4327	

Samples 58 and 57 were obtained from Wisconsin. The year these samples were harvested is not known. Sample 65 came from Iowa, harvested in 1912. Sample 68 also came from Iowa, harvested in 1915 from the Agronomy plots.

Greater encouragement was received from the resistance measurements in $KMnO_4$ than from any in conductivity water. In table I the wheat shows true correlation. The viability varies directly with the resistance. In the case of the peas (table II) the samples of from 90 to 97 per cent. germination show high resistance. With but one or two exceptions the rest of the figures for resistance fall in their proper places in the table. The experiment with timothy (table II) is not entirely in accord with the first results.

EXPERIMENT II

Corn seeds from 8 different samples were next divided into two lots. Lot 1 was placed on a window ledge overnight where the temperature reached as low as 0° F. (Subsequent tests showed that these seeds did not freeze. Hence they will be used as checks against the killed seeds).

Lot 2 was kept in a hot air oven long enough to kill the embryo. The solution resistance of these two lots appear in table IV.

In column 2 of this table, opposite the sample numbers indicated in column 1, one may find the germination percentages for the seeds in the different samples. The solution resistance before and after the heat treatment is shown in columns 3 and 4. Fig. 1 represents the results of column 3 in graphical form.

TABLE IV

SOLUTION RESISTANCE OF LIVING AND DEAD CORN SEEDS

SAMPLE	GERMINATION	SOLUTION RESISTANCE (50 MIN.)	
		Lot 1 (alive)	Lot 2 (dead)
	per cent.	ohms	ohms
155	100	19670	17395
156	99	27410	24680
157	98	17250	14760
152	96	16810	15000*
150	94	14650	12385**
148	70	12170	12980
144	65	14920	10556**
154	1	6449	5712

* Duplicate discarded. ** Browned by heat.

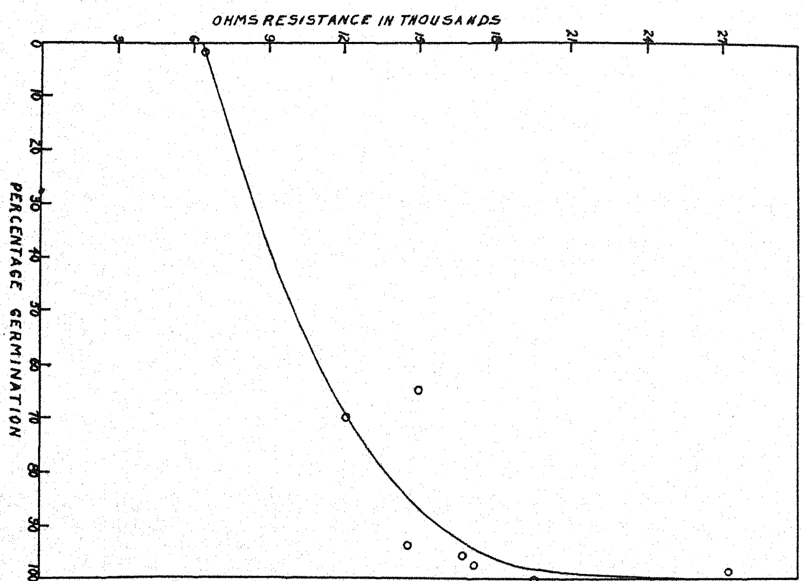

FIG. 1. Graph of relation of solution resistance to viability, from lot 1, table IV.

The samples used here were obtained from the Farm Crops Department of Michigan State College. The variety was Duncan. Eight groups were taken for the test.

Reduction of potassium permanganate by viable and non-viable seeds

In determining the solution resistance of seeds in potassium permanganate it was noticed that the permanganate was reduced at different rates

by different samples, the end-point being an easily recognized amber color. Resistance measurements taken at regular intervals did not take into account the color changes, being concerned with the changes in solution resistance only.

It was then decided to determine the time-rate of reduction of $KMnO_4$ by the seeds. One hundred corn seeds were ground to the fineness of meal, one-gram samples weighed out and permitted to stand in twenty cubic centimeters of distilled water for an hour. At the end of this time the mixture was filtered, one cubic centimeter of the filtrate drawn off and added to 0.5 cc. of M/800 $KMnO_4$. The time of adding the filtrate to the permanganate was recorded as well as the time when it was completely reduced.

The seeds were ground because the substances reducing the permanganate were dissolved in the water more readily and the process thus hastened. The cells would hardly be destroyed by this coarse grinding since it was performed in a meat chopper. Material must be ground with quartz sand in order to be reasonably sure of rupturing the individual cells. Grinding did not influence the relative rates of reduction by the different samples as was shown in several preliminary tests.

The relation of germination percentages to time-rate of reduction will be found in table V and fig. 2.

TABLE V

RELATION OF GERMINATION PERCENTAGES OF CORN SEEDS TO TIME-RATE OF REDUCTION OF $KMnO_4$ BY AQUEOUS EXTRACTS (POWDER)

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
122	98.3	38.3
124	97.0	30.8
129	96.0	40.2
128	87.5	36.7
127	87.0	29.8
126	80.4	26.8
123	34.0	13.3
121	33.0	13.0
125	3.0	5.9

It was found that, with the exception of but a few of the high-germination samples, there was direct correlation between viability and time of reduction of permanganate. The higher the germination the longer the time required for complete reduction of the permanganate.

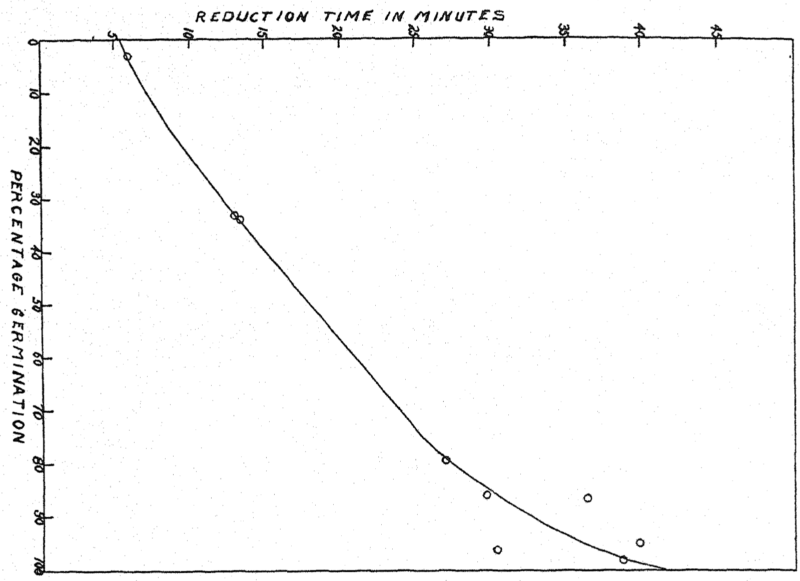

FIG. 2. Graph of relation of time-rate of reduction of $KMnO_4$ to viability, from table V.

A large number of samples was secured and the corn meal was passed through a twenty-mesh sieve before soaking. As was true in other experiments where a greater number of samples was employed, the results are not nearly so uniform. These results are presented in table VI.

TABLE VI

RELATION OF VIABILITY OF CORN SEEDS TO TIME-RATE OF REDUCTION OF POTASSIUM PERMANGANATE BY AQUEOUS EXTRACTS OF MEAL (20 MESH)

SAMPLE	GERMINATION	TIME TO REDUCE	SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.		per cent.	min.
139	100	21.9	150	94	15.9
142	100	18.5	153	93	15.5
156	99	16.5	160	93	14.1
146	99	10.9	143	83	17.9
147	99	14.3	159	79	23.5
140	99	14.2	148	70	16.5
157	98	12.5	141	71	10.3
152	96	22.5	138	65	18.3
158	96	17.9	144	65	10.9
151	95	21.9	154	1	19.0
145	94	12.0	149	0	7.5

For the purpose of shortening the method, grinding was dispensed with in another experiment and the seeds themselves were soaked in the permanganate. Ten seeds of corn were placed in twenty cubic centimeters of N/1000 potassium permanganate and the time recorded. The time was again recorded when the permanganate was completely reduced. It was found that if a few drops of N/10 oxalic acid were added to the mixture the end point would be closer and colorless instead of amber colored. The results may be found in table VII.

TABLE VII

RELATION OF VIABILITY OF CORN TO TIME-RATE OF REDUCING POTASSIUM PERMANGANATE (WHOLE SEEDS)

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
146	99	10.9
152	96	22.5
151	95	21.9
143	83	17.9
141	71	10.0
149	0	7.5

In the six samples included in table VII there is only one (146) which does not occur in the regular order. There is here almost complete correlation between viability and the reduction of potassium permanganate.

Another modification in the method was made. To hasten the time of reaction the seeds were first soaked for 12 hours in water, and 1 cc. aliquots withdrawn for the test instead of using the seeds. One cubic centimeter of this extract was treated with one drop of N/2 $KMnO_4$. The time required for complete reduction was recorded. The results will be found in table VIII.

TABLE VIII

RELATION OF VIABILITY OF CORN SEEDS TO TIME-RATE OF REDUCTION OF POTASSIUM PERMANGANATE BY AQUEOUS EXTRACTS OF WHOLE SEEDS

SAMPLE	GERMINATION	TIME TO REDUCE	SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.		per cent.	min.
155	100	23.0	167	81	20.5
161	99	23.0	168	65	19.0
162	99	21.5	171	25	12.0
163	98	24.5	173	2	7.0
172	97	11.5	169	0	18.0
164	86	24.5	170	0	12.0
166	83	21.0			

Here, too, low viability seemed to be consistent with the rapidity of reduction of potassium permanganate, although there is a great amount of non-uniformity in the table. The results in table IX were obtained in the same manner as those in table VIII. Here again the low-germinating seeds were first to reduce the potassium permanganate.

TABLE IX

RELATION OF VIABILITY OF CORN TO REDUCTION OF $KMnO_4$ BY AQUEOUS EXTRACTS OF WHOLE SEEDS

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
162	99	39
163	98	34
164	86	38
166	83	37.5
167	81	29.5
168	65	31
170	0	22
149	0	12
154	1	19
169	0	22

At the conclusion of this experiment on reduction of $KMnO_4$ by corn seeds some additional experiments were conducted with beans. The results (table X) exhibit a moderate amount of correlation. The figures for germination were obtained by selecting fifty normal-appearing seeds, sterilizing them, and testing for germination. This was done because it was learned that these were mixed samples, having been more or less adulterated by the growers. This is quite evident from the results of several different germina-

TABLE X

RELATION OF VIABILITY OF BEAN SEEDS TO TIME-RATE OF REDUCTION OF $KMnO_4$

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
174	100	31.0
175	100	31.0
176	98	25.5
177	98	26.5
178	100	19.0
179	46	38.0
180	0	14.0

tions. In one set a hundred seeds were selected at random; in another the twenty seeds that had been used in the reduction of the $KMnO_4$ were subsequently germinated. The variation observed in table XI may also be due to the difference in methods applied.

In regard to the bean seed, these were of the Navy variety and were harvested in 1925. The varying germination percentages in these samples were due to selection. The samples showing highest germination were gathered, picked, and selected after harvesting and the rest were left on the ground for several days, exposed to the climatic conditions, being sampled on different days. In this way samples of varying germination percentages were obtained from seeds that had died or were infected to seeds of high or perfect germination.

TABLE XI

GERMINATION TEST OF BEANS

SAMPLE	GERMINATION		
	100 SEEDS	200 SEEDS SELECTED	50 SEEDS, SELECTED AND STERILIZED
	per cent.	per cent.	per cent.
174	90	100	100
175	89	95	100
176	85	85	98
177	76	90	98
178	27	65	100
179	15	15	46
180	0	0	0

As will be shown in the latter part of this paper, the reducing substance has been found to be present in large quantities in the seed coat of the bean. Accordingly, ten bean seeds selected from the seven different samples were soaked over night in distilled water, and the seed coats then removed and placed in 10 cubic centimeters of N/1000 $KMnO_4$, the time for reduction being recorded. The ten seeds, after the coats had been removed, were germinated. The germination percentages and the time-rate of reduction are to be found in table XII. Not only was direct correlation between germination percentages and rate of reduction found, but sample 179, which was out of the expected order as far as time relations were concerned in table X, appeared in its proper place in table XII. This experiment was repeated at a different time with similar results.

An experiment was next performed in which the three parts of the seed (seed coat, cotyledons, plumule and hypocotyl) were treated with the potassium permanganate solution. In this case there were employed 5 seed

TABLE XII

RELATION OF VIABILITY OF BEANS TO TIME-RATE OF REDUCTION OF $KMnO_4$ BY THEIR SEED COATS

SAMPLE	GERMINATION	TIME TO REDUCE
	per cent.	min.
174	100	26
175	100	24
176	100	23
177	100	23
178	90	20
179	30	16
180	0	1

coats, 10 cotyledons, and 10 embryos. The figures for germination are those obtained from the 50 selected and sterilized seeds. The results in table XIII show almost complete correlation in all cases. The seeds germinating above 90 per cent. do not always occur in the proper order; yet they are usually higher in the table than those of low germination.

TABLE XIII

RELATION OF VIABILITY OF BEANS TO TIME-RATE OF REDUCING $KMnO_4$ BY DIFFERENT PARTS OF SEED

SAMPLE	GERMINATION	TIME REQUIRED FOR REDUCING		
		COATS	COTYLEDONS	EMBRYOS
	per cent.	min.	min.	min.
174	100	34.0	11.0	12.0
175	100	30.5	6.0	11.0
176	98	27.5	8.5	9.0
177	98	32.5	6.5	8.5
178	100	30.0	7.0	10.0
179	46	26.5	5.0	7.0
180	0	1.5	2.0	3.0

Studies of the nature of the substance

It is of interest to know the nature of this substance which reduces the potassium permanganate. REED (**22**) believes that the peroxidases in plant juices, having the power to absorb oxygen from oxygenases, will attack $KMnO_4$ in the same manner. BUNZEL and HASSELBRING (**3**) note that $KMnO_4$ may be reduced to a straw color by peroxides of manganese, and further to a clear solution by organic substances, but they hold that the oxidations are brought about by peroxides of manganese rather than by activated plant peroxidases.

That the reduction mentioned in this paper is not enzymatic may be demonstrated by first boiling the aqueous extract of seeds for fifteen minutes. The reduction will take place just as readily after boiling.

There is evidence supporting the view that these reducing substances may belong to the group of peptides, acid amides, or amino acids. If the proteins are precipitated by lead acetate and the excess lead removed by means of sodium carbonate, the filtrate will still reduce the potassium permanganate. Furthermore, an aqueous extract of the precipitate brought down by the lead acetate will not produce the reduction unless previously boiled with 10 per cent. HCl solution or incubated with pepsin solution.

Proteoses would remain in the solution after the proteins had been removed with lead acetate. However, phosphotungstic acid brought down no precipitate and it was assumed that in this case there were no proteoses present.

For a long time it was believed that ungerminated seeds contained no protein cleavage products. This view has now been changed by JODIDI (**11**) and co-workers who have found amino acids and peptides to be present in ungerminated kernels of maize, rye, wheat, and oats. BUSHEY (**4**) showed that frosted and "hailed" corn, besides being high in certain proteins, was also much higher in amide content than normal grains.

Discussion

A very detailed and extensive study of the conductance method as briefly outlined in the paper by FICK and HIBBARD (**9**) revealed that as a test for distinguishing between seeds of high, low, and medium germination it was very good. As a test for distinguishing difference in seeds varying in increments of 2 or 3 per cent. it is worthless and so far no method has yet been devised to obtain this degree of accuracy. There are some facts militating against the use of this method. It is quite expensive to begin with, and assumes that the operator has at least a technical and a more or less complete knowledge of the details of a set up for making conductance measurements.

Furthermore, the method presupposes that the electrolytic content and its nature are the same for different samples of seeds of the same species and that the change in solution resistance would be solely dependent upon the change of permeability in the seed. It is also possible that salts clinging to the seeds would affect the results although they had previously been washed with distilled water in the hope of ridding them of all salts.

The direct measurement of tissues or of any tissue as a measure of permeability has been called in question by STILES (**25**, pp. 180–182).

The work with conductance measurements in solution of $KMnO_4$ rather than distilled water offered more promising results. Though there were a

few discrepancies, resistance for the most part rose with germination percentages. Curves plotted from any of the individual tables indicate as great a degree of consistency as most workers seem to obtain from other methods. Seeds of high germination percentages repeatedly exhibit a proportionately high resistance in solution.

By far the simplest method evolved in these experiments on viability was the comparison of the time element in the reduction of $KMnO_4$ with viability. It is obvious that seeds of low viability exhibit the property of reducing the potassium permanganate in less time than is required by seeds of higher viability. It does not matter whether the seeds of zero germination have been killed by heat, frost, disease, or have died of old age. They are nearly always the first to reduce the permanganate. It is possible to employ this method for determining seeds of high, low, and medium viability, but like other methods mentioned above, it needs considerable refinement before it will show differences in seeds varying 2 or 3 per cent. in germination.

Summary

1. A review of the literature on the various methods for determining viability of seeds is presented.

2. A new and very simple method of determining viability of seeds is recommended. It is useful for seed testing and, as in other methods mentioned, in classifying seeds of high, low, and medium viability only. It is not acceptable when difference in germination of two or three per cent. is desired, and we have still to hunt for a means of obtaining this degree of accuracy. The method suggested consists in determining the time-rate of reduction in $KMnO_4$ solution in which the seeds are soaking.

3. Proof is furnished that the substance which reduces the $KMnO_4$ is not an enzyme and that it may belong to the group of substances known as amino acids, peptides, and amides.

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BRIEF PAPERS

PLANT PHYSIOLOGY IN RUSSIA

(WITH TWO FIGURES)

In visiting the principal agricultural experiment stations and universities on a trip through European Russia during the summer of 1927, the author was impressed by the especially advantageous position of the plant physiologists there, and with the fine quality and great extent of the research in progress. American physiologists have known of the high regard among Russian people for TIMIRIAZEFF and PALLADIN. Through the accomplishments of these men and of contemporaries, plant physiology enjoys a standing which may well be emulated by us. There is no tendency to place this subject in a subordinate position, as is so frequently done in America, where physiological research is often under the direction of a pathologist or crop specialist with strong political or economic connections. The Russian physiologist is concerned with solving fundamental principles in physiology and is by no means held to the solution of problems of immediate commercial application or routine work as many plant physiologists are in the United States.

Work on respiration and upon enzyme action is being prosecuted by Dr. S. KOSTYTSCHEFF with a staff of research workers in spacious and well-equipped laboratories of the Academy of Science in Leningrad. The annual budget for supplies and equipment might be envied by most of our experiment station physiologists. Work on photosynthesis seemed everywhere to be in progress, and well equipped. Dr. N. A. MAXIMOW and Mme. KRASSNOSELSKIA-MAXIMOW had extensive equipment at the Detsko Selo station. (See fig. 1.) They had just completed an important phase of their work on the photosynthetic rate. At the Botanic Garden in Leningrad Dr. LUBIMENKO with several assistants was conducting work on this same problem and upon the quantitative relationship between chlorophyll content and photosynthesis. He has devised an excellent spectrocolorimeter method for the quantitative determination of chlorophyll. Both of these laboratories were equipped for the control of light, temperature, and humidity. Students from these laboratories were busy also at the biological station at Alexandrovsk on the Arctic Sea.

The physiological laboratories of the Institute of Applied Botany at Detsko Selo under Dr. MAXIMOW are well equipped also for work on root physiology and development. Mme. HELEN KRASSOVSKY is principally concerned with this phase. Work on drought resistance is of major impor-

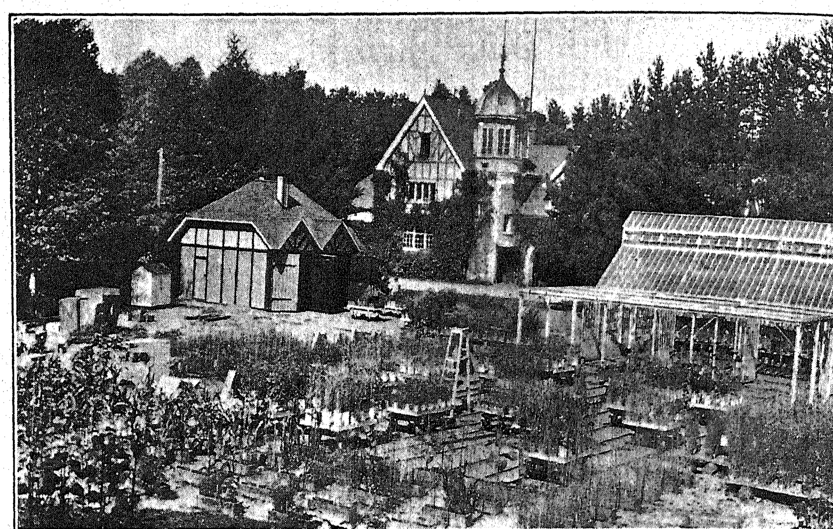

FIG. 1. Dr. MAXIMOW's experimental greenhouses, plots, and photoperiodism work, and drouth resistance experiments. Photograph by HARVEY.

tance in Russia. Dr. MAXIMOW and Mr. IVAN TUMANOV were giving much time to it. Work on the low temperature resistance of plants was being greatly increased through the installation of three refrigerating machines of the Audfren Singrun type, and by the construction of low temperature greenhouses. Miss LEBEDINZEV was working especially on the dilatometer method of determining free and bound water in plants. Work on photoperiodism at Detsko Selo had close correlation with the cytological work of Dr. LEVITSKY. All of the physiological work enjoys the best of relations with the plant breeding department under Dr. PISSAREFF, and with the soil nutrients work of Drs. GLINKA and KASANOFF. (See fig. 2.)

Field stations for supporting the work on frost resistance, drought resistance, and photoperiodism have been established all over Russia from west to east, and from the Black Sea to Kola. These are established at short intervals of latitude and give a great range of frostless period, length of day, and of other climatic conditions. An unparalleled series of meteorological observations are taken at each station. It is to be regretted that meteorological stations in the United States are not as well correlated with such work in agriculture. The meteorological stations in the Hibini Mountains determine all the weather conditions at five altitudes up to 3,000 feet. The instruments are of the most recent type and readings are checked by two or three kinds of instruments, thus giving a comparison between types of record for the same ecological factor. At Hibini ten meteorologists were on duty.

FIG. 2. Dr. KASANOFF and the mineral nutrient experiments at the Agricultural College at Detsko Selo. Photograph by HARVEY.

The work of the Institute of Applied Botany under the active leadership of Dr. VAVILOV has concerned itself greatly with the physiological problems related to plant geography and plant introduction.

One gets the impression of a thoroughly conscientious and helpful governmental support for fundamental research throughout Russia. The lack of organization of the work in plant physiology and geography in our own Bureau of Plant Industry is certainly in strong contrast with the fine conditions given by the Bolsheviki for these phases of research. Very few of the scientists in Russia are Communists, yet their work is given excellent support. The scientist is left alone by the politicians. If our government is ever to give adequate support to the fundamental and necessary work in plant physiology, the research organizations supported by the government must likewise be entirely free from political and unsympathetic management.—R. B. HARVEY, *Cambridge University, England.*

SOIL MOISTURE AT PERMANENT WILTING OF PLANTS

The residual moisture in the soil when plants attain the condition known as permanent wilting has been the subject of much work and discussion among those interested in plants and soils. BRIGGS and SHANTZ held that when a given soil reached a certain moisture content, plants growing thereon wilted, and could not be revived until additional water

was added to the soil. CALDWELL, and SHIVE and LIVINGSTON, disagreeing with this conception, held that permanent wilting depended upon atmospheric evaporating conditions. BRIGGS and SHANTZ further held that the wilting coefficient of a given soil could be calculated from the moisture equivalent without the necessity of a direct determination by actually wilting the plants. As a result of some 1300 trials with 20 soils they reached the conclusion that the wilting coefficient could be obtained by dividing the moisture equivalent by the factor 1.84.

In extensive experiments extending over a period of years, the writers have observed a remarkable constancy of the residual moisture content for a given soil when permanent wilting is attained under widely varying evaporating conditions. While most of the work was done with sunflowers, a number of trials with other plants seemed to substantiate the contention of BRIGGS and SHANTZ that on a given soil all plants reduce the moisture content of the soil to about the same extent when permanent wilting is attained. In an earlier report[1] the writers showed that with some soils there was a remarkable agreement between the observed and the calculated wilting coefficient. However, later results with many kinds of soils do not uphold the correctness of the 1.84 ratio, and we do not believe that a common factor for all soils may be used to calculate the amount of water which remains in the soil at permanent wilting.

The plants were grown in two sizes of containers under the varied seasonal atmospheric conditions which prevail at Davis, California. Maximum temperatures varied from about 45° F. during the winter months in an unheated greenhouse to 115° F. out-of-doors during the summer. Evaporation during these periods from a white spherical atmometer varied from 4 or 5 cc. to 80 cc. for 24 hours. A full description of the experiments will be published later. Some of the results obtained in over 2000 trials with 29 soils are given in the acompanying table.

It will be seen at once that the ratios range from 1.73 to 3.82. The results show clearly that there is no relation between the ratios obtained and the type of soil used. No logical grouping is possible because some of the highest as well as some of the lowest ratios were found within a given classification of soils. For example, high and low ratios were found with sands, with loams, and with clays. It would thus seem from the data obtained with the soils and plants used in these experiments that the residual moisture at permanent wilting cannot be obtained in every case from the moisture equivalent by use of the factor 1.84.

[1] VEIHMEYER, F. J., and HENDRICKSON, A. H. Soil Moisture conditions in relation to plant growth. Plant Physiol. 2: 72–81. 1927.

TABLE I

RESIDUAL MOISTURE AT PERMANENT WILTING AS DETERMINED WITH SUNFLOWER PLANTS OCTOBER, 1927, TO JUNE, 1928, AT DAVIS, CALIFORNIA

SOIL	NUMBER OF TRIALS	*MOISTURE EQUIVALENT	* PERCENTAGE OF MOISTURE IN SOIL AT PERMANENT WILTING	RATIO OF MOISTURE EQUIVALENT TO MOISTURE AT PERMANENT WILTING
N	18	3.29 ± 0.022	1.41 ± 0.031	2.33 ± 0.054
U	16	6.05 ± 0.036	3.50 ± 0.030	1.73 ± 0.018
HS–2	35	8.32 ± 0.033	3.67 ± 0.020	2.27 ± 0.015
HS–1	35	8.84 ± 0.041	3.99 ± 0.030	2.295 ± 0.020
FS	226	10.50 ± 0.026	3.08 ± 0.007	3.41 ± 0.011
TL	78	13.71 ± 0.021	4.51 ± 0.020	3.04 ± 0.014
J	17	17.07 ± 0.017	6.15 ± 0.070	2.775 ± 0.013
Y	40	17.16 ± 0.033	8.82 ± 0.027	1.95 ± 0.007
TC	24	17.30 ± 0.044	7.89 ± 0.040	2.19 ± 0.012
S	39	21.35 ± 0.043	10.20 ± 0.029	2.09 ± 0.007
OL	27	23.36 ± 0.052	6.12 ± 0.033	3.82 ± 0.022
OC	29	24.51 ± 0.050	11.55 ± 0.059	2.12 ± 0.012
MG	151	25.63 ± 0.039	10.47 ± 0.025	2.45 ± 0.007
V	24	37.90 ± 0.067	19.03 ± 0.074	1.99 ± 0.008

* Calculated on a dry weight basis.

While the residual moisture content of some soils at permanent wilting shows close agreement with the 1.84 ratio, as the writers have previously shown, the general use of this ratio for all soils is open to serious criticism. From the foregoing data, it is evident that the amount of water available for plant growth cannot be determined from the moisture equivalent alone. In the opinion of the writers, the moisture equivalent is the best single-value determination for interpreting the moisture properties of soils, but it is not an exact measure of how much of that water is available to growing plants. For accurate work it is evident that the amount of readily available moisture can be obtained only when the amount of residual moisture at permanent wilting is known, because it seems that plants are able to reduce the moisture content of different soils to different degrees of dryness before this stage of wilting is reached.—F. J. VEIHMEYER AND A. H. HENDRICKSON, *University of California.*

NOTES

Officers for 1928–1929.—The Secretary of the American Society of Plant Physiologists has announced the results of the recent annual election as follows: For President, Dr. E. J. Kraus, University of Chicago; Vice-President, Dr. Scott V. Eaton, University of Chicago; Secretary-Treasurer, Dr. H. R. Kraybill, Purdue University. The Society is in position now to go forward with great confidence in the future. The budget for the publication of Plant Physiology has been balanced during the last two years, and the normal growth should continue at a rate sufficient to provide for enlargement of the publication program from time to time. All members will want to cooperate fully with the officers in planning for the New York meeting, and a period of rapid advancement is anticipated for the coming year.

Sixth National Colloid Symposium.—The sixth National Colloid Symposium of the American Chemical Society was held in Canada at the University of Toronto on June 14–16, 1928, with Sir William Hardy, of Cambridge, England, as the guest of honor. About 150 members registered, and there was a large number of visitors from Toronto at the meetings. Twenty-six papers were scheduled for the three days.

Sir William Hardy, in his opening address, spoke of the relation of colloidal behavior to biological problems, asserting his belief that the next great advance in science would be in the field of biology.

There were few papers of direct interest to plant physiologists, both soil and plant colloids being rather neglected. The program, however, was varied, and brought into prominence the wide range of colloidal applications. Electrical relations were dealt with in several papers, but Dr. Harkins was unavoidably absent, and unable to introduce the topic. Adsorption, at the hands of Professors McBain and Bancroft, was given a considerable amount of discussion, Dr. Bancroft amusing his hearers with his well-known aversion to "activity." Rubber, medicine, bacteria, explosives, and photography were among the subjects which received attention. Possibly of greatest interest to the plant physiologist was the paper by Professors Wastenys and Borsook, of the University of Toronto, on the enzymatic synthesis of protein, with its suggestion of a method by which different proteins are synthesized *in vivo*. The utilization of moving pictures in ultramicroscopic fields was a noticeable feature of the program.

The city of Toronto provided many points of interest. At the University, the Connaught Laboratories and the science buildings were open to inspection. Among the experiments shown was a successful run for the

liquefaction of hydrogen and helium. For entertainment, golf was arranged on a variety of courses, and the swimming pool in Hart House was an attraction. The annual banquet was held on Friday in the Great Hall, and few will ever forget the delightful costumes and the excellent music of the sextette of "Canadian Singers" after the dinner, and later in Hart House theater.

International Nitrogen Conference.—The second International Nitrogen Conference was held on board the Steamship *Luetzow*, on the Adriatic Sea, May 1–5, 1928. It was sponsored by the nitrogen fertilizer interests of France, Italy, England, Norway, and Germany. Some well-known names appear among the speakers and attendants at the meeting. Addresses were made by Sir FREDERICK KEEBLE, Dr. ERWIN BAUR, Prof. L. BRETIGNIÈRE, Dr. A. DEMOLON, Dr. J. BUEB, Prof. FIRMAN E. BEAR and others. Participating in the discussion were such noted figures as Sir DANIEL HALL, and Prof. HENRY ARMSTRONG, of England. Fifteen countries were represented. With the discovery of processes for making synthetic fixed nitrogen cheaply, a vast nitrogen fertilizer industry has sprung up which is destined to play an important part in the permanent fertility of agricultural lands. It is also probably destined to figure prominently in the commercial field, with a tendency toward vast industrial monopolies, with international understandings and agreements as to distribution and prices of nitrogen fertilizers. The commercial significance of the meeting probably outweighs its scientific significance.

Seeds For Investigational Purposes.—Since much work needs to be done on the physiology of trees, sources of tree seeds are important to the investigator. The seeds of many native trees and woody shrubs exhibit delayed germination. Otto Katzenstein and Co., 6 Cone St., Atlanta, Georgia, handle seeds of many of our native trees and shrubs, and the seeds of such cultivated fruits as apples, apricots, cherries, lemons and oranges, pears, persimmons, plums, and quinces. They also carry roots and seeds of medicinal plants. Thirty years of experience in this field makes possible excellent service to those who need seeds for experimental purposes.

Books of Historical Value.—Occasionally one finds it desirable to obtain books which have historical value. Not long since it was possible for the writer to secure a good copy of JETHRO TULL'S Horse-Hoeing Husbandry, 1733, from THOMAS THORP, Strathfieldsaye, Guildown Road, Guilford, England. This work had been sought for several years without success. Others who desire old books might find it worthwhile to submit want lists to Mr. THORP.

Summer Meeting at Purdue University.—Plans for the summer meeting at Purdue University have been formulated by the Secretary-Treasurer in cooperation with the Purdue Section. On September 4, a number of papers will be presented by members of the Purdue Agricultural Experiment Station, dealing with the work of interest to plant physiologists which is now in progress at the station. The following day will be devoted to field excursions and demonstrations. The dinners and luncheons will be made periods of delightful social intercourse, and visitors are assured a most valuable experience. It is hoped that many of the members will find it possible to participate in this meeting.

The Fifth Annual Meeting.—Plans for the fifth annual meeting of the American Society of Plant Physiologists at New York in December, 1928, are rapidly maturing. The program committee is hard at work on the various features of the meeting. The members of the committee should be given hearty support by everyone, and this fifth annual meeting should be made the largest and best meeting in our history. Since active and intelligent discussion of the papers presented adds pleasure and zest to these occasions, those in attendance can help to make the meetings most valuable by engaging in such discussions. The programs should not be so crowded that discussion is precluded. It should also be remembered by those who offer papers to the program committee, that attendance and personal presentation of the papers listed is a courtesy to the program committee and to the Society which should not be omitted. Visitors are sometimes keenly disappointed when papers have to be read "by title only." If attendance is doubtful, it is better not to list papers on the program.

The Stephen Hales Prize.—The committee which was appointed to devise a method of handling the STEPHEN HALES Prize has practically completed its work, subject to the approval of the executive committee, and acceptance by the Society. The committee which is to select the first recipient of the award consists of the following members: Dr. J. B. OVERTON, University of Wisconsin, chairman; Dr. A. L. BAKKE, Iowa State Agricultural College; and Dr. C. R. BALL, of the U. S. Department of Agriculture, Washington, D. C.

Life Membership Committee.—The committee chosen to select the third CHARLES REID BARNES life member has been appointed by the president. The personnel of the committee is as follows: Dr. W. M. ATWOOD, Oregon Agricultural College, Corvallis, Oregon, chairman; Dr. P. D. STRAUSBAUGH, West Virginia University; Dr. E. B. MAINS, Purdue University; Dr. A. E. MURNEEK, University of Missouri; and Dr. H. W.

BROWNING, Rhode Island State College. This committee has one of the most pleasant duties to perform. The report of the committee will probably be made at the annual dinner at the New York meeting.

Membership Committee.—The membership committee for the year 1928–1929 has recently been appointed, and consists of the following members: Chairman, Dr. E. M. HARVEY, Oregon Agricultural College; Dr. A. L. BAKKE, Iowa State College; and Dr. EARL S. JOHNSTON, University of Maryland. Rapid growth can be made if all members assist the committee in its important work. If you know of someone who should be invited to membership, send the name to some member of the committee, or to the Secretary-Treasurer, Dr. H. R. KRAYBILL, Purdue University, Lafayette, Indiana.

Starch Chemistry.—A symposium on the chemistry of starch, and a bibliography of nearly 10,000 titles on this subject has been brought together in book form by ROBERT P. WALTON in collaboration with a group of authors of international reputation. There are nineteen papers in the symposium, some of which deal with the molecular constitution of starch. The first four sections consider thermal depolymerization, methylation, enzyme hydrolysis, and bacterial degradation, with reference to these problems of molecular constitution. Other papers consider the colloidal properties, X-ray spectrography, nature of amylases, rôle of starch in bread making, conversion in the fermentation industries, manufacture of corn and potato starch, production of dextrins and gums, and other adhesives, and the use of starch in the paper and textile industries. The final section considers the historical development of starch chemistry and manufacture. While the price of the book is $10.00 the bibliography is an expensive item in the cost of publication. It is published by the Chemical Catalog Co., New York.

Principles of Plant Physiology.—A new text-book of plant physiology has appeared from the press of the Macmillan Co. The author, Dr. ORAN L. RABER, best known for his interest in permeability, is at present a member of the staff of Biological Abstracts.

The book is intended for elementary classes, but somewhat overshoots the mark so far as the chemistry is concerned. There are thirty-one chapters, some of which are well done, while others are not satisfactory. The order of arrangement is not so very logical. For instance, after the introductory chapters on the sciences and the cell, come chapters on photosynthesis and nitrogen metabolism, and the chemistry of carbohydrates, fats,

waxes, lipides, proteins, miscellaneous products and enzymes, preceding the more readily grasped processes of water absorption, diffusion of gases, osmotic action, transpiration, etc. Aerobic respiration precedes anaerobic in the treatment, although in the actual process it is pretty certain that it starts anaerobically, and becomes aerobic in such organisms as possess the necessary enzymes. The last two chapters have little to do with plant physiology, and the work would not suffer by their omission. The main criticism to be made is the large number of errors. Many of them are small, but it is disappointing to find errors of statement, or errors of judgment, sprinkled freely through the work. Beginning students do not have the necessary background to detect these mistakes, so that the instructor who uses the text will have to watch for the errors involving emphasis, points of view, and essential facts. The need of a good text in this field is very urgent, and RABER is to be complimented for having made the attempt to provide a text. The price of the text is $3.00, and the book can be ordered from the publishers, Macmillan Co., New York.

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GERMINATIVE ENERGY OF LOTS OF CONIFEROUS-TREE SEED, AS RELATED TO INCUBATION TEMPERATURE AND TO DURATION OF INCUBATION[1]

FERDINAND W. HAASIS

(WITH FIFTEEN FIGURES)

Introduction

The rate at which heat passes into or out of the plant body varies continually and the temperature of the plant always tends to approach that of the surroundings, from which it usually differs but slightly. The temperature of the surroundings may consequently be considered as an index of the concurrent plant temperature, and environmental temperature (as of soil and air) is usually looked upon as one of the chief influences that determine physiological activity. It is becoming increasingly appreciated that the influences exerted by temperature upon organisms are themselves dependent, however, on concurrent influences of other kinds. Some of these non-temperature influences are primarily related to, or bound up in, the internal characteristics of the organism or tissue considered, while others are active more or less directly at the external periphery of the organism and consequently operate inward from the environment. Whether any individual seed may germinate at all, for example, or how rapidly the physiological processes of germination may occur, with a specified set of temperature conditions, clearly depends only partly on the temperature complex itself, for the kind of seed considered, its physiological condition, etc., must obviously be important among the controlling conditions. The germination response of any viable seed to a given temperature complex is dependent upon many environmental conditions other than those of temperature; with a temperature complex that would otherwise give rapid

[1] Botanical contribution from the Johns Hopkins University, no. 104.

germination, this process may be greatly retarded or even wholly inhibited if the rate of water supply or of water loss, the rate of oxygen supply, or the rate of supply (or of removal from the surrounding region) of poisonous or stimulating materials (such as salts and organic compounds) is either too high or too low for satisfactory development of the organism when under the influence of the particular temperature complex in question. This recently familiar line of thought leads inevitably to the proposition that no statement about the temperature relations of vital processes can be more than very superficially useful, in regard to the definite analysis of physiological control and causation, unless the concomitantly effective, non-temperature conditions as well as those of temperature, are adequately defined. Of course the definition or description of these background influences cannot as yet be very precise in many respects, but they need to be and they can be clearly enough set forth to make it possible for other experimenters to approach them to an adequate degree.

Any given environmental temperature complex has several characteristics. The intensity or degree of the temperature obtaining in or around the organism considered (conveniently expressed as degrees on a thermometer scale) is first thought of; we may say that a seed germinated at a temperature of 25° C. But the temperature of a germinating seed is seldom even approximately maintained for any considerable period of time and temperature fluctuation consequently requires special consideration. Temperature may fluctuate very little (being nearly maintained) or it may fluctuate greatly and the fluctuations, whether small or large, may be slow or rapid in regard to time, often differing in rate for different portions of the experiment period. As has been emphasized by MacDougal (**14**), if the temperature is not practically maintained the form of the thermographic curve or tracing for any time period may be fully as important physiologically as are the corresponding minimum and maximum temperature values. Finally, whatever temperature complex is dealt with, whether the temperature of the organism is nearly maintained or fluctuates according to some adequately described pattern, the duration factor is of course of prime importance. Any statement of a physiological temperature relation must be considered as holding only for the exposure period for which that statement is made. We may say that a certain seed germinated with a maintained temperature of 25° (with regular or irregular fluctuations of plus or minus 1°, for example), but our statement is without much value unless we add that germination occurred within a certain number of hours, days, etc., following the beginning of incubation. Some of these considerations of the nature of temperature influence on organisms have received attention from Fawcett (**6**).

Technique and rational analysis have not yet progressed sufficiently to encourage the undertaking of serious experimental studies on the influence exerted on physiological processes by different forms of temperature fluctuation, and most of the investigations thus far reported in physiological literature have dealt with approximately maintained temperatures. From the point of view of ecological science (including forestry, agriculture, hygiene, etc.), for which natural conditions must be studied, temperature change has received some attention, but before the influence of fluctuating temperature can be approached by analytically planned experimentation it will be necessary that our knowledge of the much simpler maintained-temperature relations of organisms and tissues be greatly increased.

Many aspects of plant temperature relations are of great importance in plant ecology and the applied sciences that rest on plant physiology. This is especially true in regard to seed germination, for the germinating seed is usually so intimately and simply related to its surroundings that its temperature relations offer excellent opportunities for experimental study and for the application of physiological principles. Also from the standpoint of physiology itself seed germination is of exceptional interest, for it presents fewer difficulties than most of the other phases of plant activity and the complexity of its relations is correspondingly less discouraging to the experimenter. Furthermore, seed germination forms the starting-point for many different kinds of experiments upon the conditions that influence other plant processes, and precise knowledge and exact appreciation of the causal controls of germination are required for the working out of standard treatments calculated to produce a number of physiologically similar plants for experimental study. Experimentation on any aspect of plant activity is not apt to advance either rapidly or far until it is possible to secure at will lots of plants that are nearly enough alike for the special requirements of the investigation in hand. It may be added that the germinating seed furnishes us with a very compact and relatively simple vital system, which embraces most of the essential physiological processes. Seed germination and the growth of young seedlings have always been a favorite field for experimentation of both the cruder and the more refined types.

Comparatively few studies have been made on the temperature relations of seed germination from the standpoint of the fundamental analysis of determining conditions or controls, and the work here reported was planned to throw a little additional light into this particular corner of plant physiology and to furnish some aids and sign-posts that may be more or less useful to future workers who may wish to explore this field more thoroughly. From the nature of the problems involved, this study is of course of only

preliminary and exploratory nature. The concrete or immediate object was the determination of the influence of maintained temperature, acting for various lengths of time, upon the germinative energy of lots of seed of several different species of coniferous trees, especially pitch pine (*Pinus rigida* Mill.), the percentage of germination occurring under the test conditions being considered as the index of germinative energy.* A fairly extensive study of rice seed (*Oryza sativa* L.) was also made and seed of other agricultural plants was studied to some extent, but only the results obtained with seed of certain pines and other conifers will be presented in this publication.

It was especially desired to find out the optimal temperature for the occurrence of a high percentage of germination under the general conditions of the experimental tests and with different lengths of the incubation period, but attention was also paid to minimal and maximal temperatures and to the forms or patterns of the temperature-germination graphs. An attempt was made to employ more or less standard treatments of the seed samples with regard to the many influential conditions aside from temperature and the duration of the incubation period, but the aim of the study was not to find out just how to specify an environmental complex that would produce high percentages of germination in various lots of seed. That would constitute a practical problem that might promise the possibility of more immediate application in forestry and agriculture, but it might prove to be too complex for attack until a number of much more restricted and scientifically fundamental questions had been already answered much more clearly than is now possible. Indeed, the present study may be considered as an attack on one, or a very few, of the great array of such fundamental questions that must occur repeatedly to every student of physiology and the related applied sciences. It is hoped that the non-temperature conditions of the environment that were effective in these experiments are clearly enough described, in terms of the technique by which they were secured, so that other experimenters may be able to approach them to a fairly satisfactory degree. For some maintained temperatures and for some lengths of incubation period, the technique used surely furnished very satisfactory environmental complexes for the germination of large proportions of the main lots of seed studied.

Several matters of definition and logical analysis deserve attention at the outset. Following the usage of seed testers, *germination percentage* or the *percentage of germination* denotes the average number of seeds that germinated per hundred seeds used in any test. A sample of seeds is ex-

* This use of the word *energy* may be confusing, since the word is not here employed in its physical sense, but "germinative energy" has been used in discussions of seed testing and is recommended by TOUMEY (22).

posed to the influence of an environment calculated to induce germination in some of them at least, the number found to have germinated at the end of a certain period of time is ascertained, and this number is expressed as a percentage of the number of seeds in the sample used. For any test, this numerical value is considered as an index of the germinative energy of the sample tested and, if the sample fairly represented the entire lot of seed from which it was taken, the germinative-energy index of the sample is taken as the germinative-energy index of the lot. Several samples of any lot of seed are generally subjected to like tests and the germinative-energy index of the lot is the average of the indices of the samples. On the basis of any particular kind of viability tests a given lot of seed is to be considered as made up of two categories of individuals, (A) those that are capable of germination and (B) those that are not. If, for example, ninety per cent. of the seeds in a lot are individually viable for the kind of test used we say that the viability of the lot is 90 per cent. on the basis of that kind of test, or that the percentage viability of the lot is 90, and that value is the index of the germinative energy of the lot for the particular environmental complex used in the tests from which it is derived.

The viability of a lot of seed thus exhibits a special kind of variability, with reference to the innate capacity of the lot to produce seedlings, and with reference to a specified set of environmental conditions. This concept is not to be confused with those of growth rate, respiration rate, etc., as such process rates are dealt with in physiological literature. The lot index of viability does not represent a rate, it represents merely the proportion of the seed population that are individually viable under the conditions of the test. Process rates, on the other hand, such as the rate of elongation of seedling roots, for instance, have not been generally studied with primary regard to the innate variability of the lots of seedlings or plants studied. Indeed, most experimenters with process rates subject their experimental material to somewhat careful selection before each series of tests is begun, the aim being to secure as high a degree of uniformity in seeds, seedlings, etc., as is possible. Thus, seeds are often selected for uniformity in size, color, weight and specific gravity, and seedlings are frequently selected for size, form, color, for previous rate of growth or other physiological capacity, etc. Such preliminary selection is essential in studies on the environmental control of physiological processes, as every one knows, for the results of such studies are valid only for the particular internal conditional complex that was represented by the lot of plants for which the results were derived. Because satisfactory uniformity in any lot of material is difficult to secure, it remains desirable, however, to give special attention to variability, even after uniformity has been approached as nearly as possible by various kinds

of preliminary selection. It is this thought that has led to the employment of elaborate statistical methods for the examination of experimental results that lend themselves to such treatment. Innate variability implies internal differences in physiological capacity, and hence in performance or behavior, among the several individuals of an experiment, and it constitutes at present perhaps the most serious obstacle to physiological and ecological progress. This statement applies especially to the logical analysis that is so important in the planning of experimental procedure as well as in the interpretation of experimental results.

Since innate variability is to be reduced as much as possible in experimentation on physiological control and since it is always present, in spite of everything an investigator may do, it becomes itself an important and very fundamental characteristic of any group of organisms, such as seeds or seedlings, cuttings or scions, etc. It requires attention and measurement at every turn. But variability is not a characteristic of the individual unit in an experimental series; it is of course a characteristic of the group only. In the sense here considered a single seed, for example, does not manifest variability. An individual seed possesses size, color, a certain amount of capacity for germination under any set of environmental conditions, etc., and the innate variability of a sample of seeds, however it may be measured, depends on the manner in which the several individuals of the sample differ from one another; an index of variability must characterize the physiological differences that obtain in the group. Knowledge of this group characteristic should permit selection for uniformity with regard to the several kinds of variability and should facilitate the interpretation of the numerical results of experiments on physiological processes. As has just been implied, such knowledge is also desirable whenever different lots of individuals are to be compared, as in ordinary seed testing, for example. The aim of the seed tester is not primarily to ascertain just how individual seeds differ, but how one lot of seed differs from another lot with respect to capacity to produce seedlings. Individual differences are studied primarily to obtain information by which the lot differences may be compared.

An index of germinative energy needs always to be stated with specific reference to the particular complex of environmental conditions with which the germinative-energy tests were made, for the index value cannot be considered as valid except for that complex. It is clear that the numbers of seeds germinating in two like samples and the germination percentages derived therefrom may be very different if the two samples are tested in different ways. Water conditions, oxygen conditions, temperature conditions, etc., are all influential, and especially the duration of the test period. For any lot of seed, for any set of non-temperature conditions and for a

stated length of the test period, the index of germinative-energy may have widely different values according to the temperature conditions of the test; for any lot of seed and for a stated set of physical and chemical conditions in the test, the value of the index of germinative energy may differ greatly according to the length of the period of incubation, etc. In short, the magnitude of the germinative-energy index is a function of a large number of variables, of which only a portion represent characteristics of the lot of seed considered, while the rest stand for the environmental conditions of the test and its duration.

Because of these considerations the germination percentage derived from any test of a sample of seeds is to be regarded only as corresponding definitely to the conditions and duration of the test. The index value may have any magnitude between zero and a maximum, the upper limit being determined by the characteristics of the lot of seed in question. It is consequently essential that we try to understand what are the environmental influences that affect the index value for any lot of seed and just how they operate to give that value any particular magnitude. Such an investigation as is here suggested may be approached only by means of analytically planned experimentation. For the present, most of the experimental conditions have to be relegated to the category of background conditions (to be described as adequately as possible and made uniform throughout the several tests of an experiment) while a very few may be considered, in any case, as experimental variables (to be studied with regard to differences in their intensities and qualities and always with special reference to the chosen background complex).

In the experiments dealt with in this paper all influences were in the background excepting (1) lot of seed, (2) maintained temperature and (3) duration of incubation. It will be seen in this report, for example, that the first lot of pitch pine seed tested gave, for the standard set of background conditions employed, many different indices of germinative energy, ranging from zero to about 90, the index values being environmentally influenced (always with specific reference to this particular set of background conditions) by temperature and duration of incubation. For shorter periods of incubation (not too short) the values are all low but they are higher with some maintained temperatures than with others. For longer periods of incubation they are very high with some maintained temperatures and very low with others. Whether the highest percentage of germination shown approaches the physiological and statistical limit for that lot of seeds remains questionable, but (since the highest value is close to 100 per cent.) it is clear that the maximum viability percentage was nearly attained.

An interesting feature of these results is the manner in which the form of the temperature-germination graph changes with the length of the incubation period, and it appears that the innate nature of any lot of seeds cannot be well understood unless this feature is experimentally studied. The optimal maintained temperature for the occurrence of germination in a given sample of seeds (or the temperature giving the highest index of germinative energy for that sample and for the test conditions) is not at all the same for short incubation periods as for longer ones, unless all periods are relatively long.

The individual seeds of any lot differ as to germinating power, and experimentation of the sort employed in these studies furnishes data for classifying the individual seeds in this respect. The results show what percentage of the lot (how many per hundred, on the average) may be expected to germinate under the influence of each set of test conditions (the duration factor being considered as one of the test conditions) and also what percentage of the lot may be expected to fail to germinate under the influence of each set. Failure to germinate does not necessarily imply lack of viability in a seed. Seeds that fail to germinate under one set of conditions may of course germinate under another set. For any given set of test conditions and for any incubation period, the seeds of any sample may thus be classified into germinable and non-germinable. For any set of non-temperature conditions and for any incubation period they may be classified further into several categories with regard to temperature. This question of the experimental classification of a lot of seeds is referred to in a subsequent section.

Different lots of seed are of course to be expected to differ in regard to statistical make-up, in spite of their being of the same species or variety, from the same individual plant, from the same inflorescence or even from the same capsule. Such differences between lots of seed, which are the differences with which a seed tester deals, are partly due to differences in the original proportions of "good" and "bad" seeds when the lots were harvested. Also, since stored seed is apt to deteriorate with time, the germinative-energy index of a given lot (for a particular kind of test) may alter more or less rapidly with time, according to the original make-up of the lot and the conditions of storage.

The analytical study of the temperature relations of vital phenomena has been approached by various methods and this whole field of science was reviewed, up to 1897 and 1914, respectively, by DAVENPORT (**3**) and KANITZ (**8**). A number of studies on the temperature relations of plants have dealt with the rate of elongation of roots and shoots of seedlings after germination has occurred. Examples are afforded by the studies of SACHS

(**19**), KOEPPEN (**9**), DE VRIES (**4**), LEHENBAUER (**11**), ISABELLA LEITCH (**12**) and NEWCOMBE (**15**). Since the studies considered in the present paper do not deal directly with rates of growth, they are not closely related to the contributions here cited and it is not necessary to present in this place a résumé of the latter.

Some investigators have studied the germinative energy of lots of seed as indicated by the percentage of germination when samples of each lot were exposed to the same or different environmental complexes for various incubation periods. Studies of this kind are somewhat more closely related to the present studies than are those dealing with rates of shoot or root enlargement, but the earlier results offer but little opportunity for analytical comparisons with those set forth in the present publication. Productive comparisons are generally precluded by the nature of the plans of experimentation that were followed. In this group may be mentioned reports by NOBBE (**16**), RAFN (**18**), DOROSHENKO (**5**), PACK (**17**), HARRINGTON (**7**), SIFTON (**21**), and especially those by BOERKER (**2**) and KORSTIAN (**10**).

NOBBE (**16**) aimed to find out what are the most suitable temperature conditions for germinative-energy tests of certain forest-tree seeds. He employed maintained temperatures and also studied the influence of alternations of maintained temperature. In the latter case a sample of seeds was exposed to the action of two different maintained temperatures, for different parts of the incubation period. His background conditions (considered as uniform for all cultures) were those of moisture and oxygen supply and his variables were temperature, length of incubation period and kind of seed. These studies were of course planned for practical application in seed testing rather than for the analysis of physiological control. The studies of HARRINGTON (**7**) were similar to those of NOBBE.

RAFN (**18**) aimed to have as background conditions all influences excepting kind of seed and duration of the incubation period. His studies included lots of seed of more than two hundred different tree species. DOROSHENKO (**5**), PACK (**17**) and SIFTON (**21**) employed as variables maintained temperature, duration of the incubation period and kind of seed.

BOERKER (**2**) studied the germinative energy of a number of kinds of tree seeds and considered as variables the kind of seed (species, race, size, etc.), the duration of the incubation period, the intensity of illumination (natural light modified by shade, in a greenhouse), soil-moisture content and soil texture. He gave little attention to temperature, however, and his air-temperatures, above the seed bed, fluctuated in irregular manner, between about 8° and about 38° C., when measured in the shade. The extent of the weekly temperature range was never less than 16° and it was

sometimes as great as 29°. His experiments lasted about six months and he regarded the temperature conditions as optimal throughout that period. The results are presented as graphs, in which ordinates represent percentage of germination and abscissas represent the length of the incubation period expressed in days.

KORSTIAN (**10**) studied the germinative energy of acorns of several species. His variables were the kind of seed (species), the duration of the incubation period (measured in five-day units) and the temperature conditions, other influences being about uniform for all tests and having values not far from what he regarded as optimal for the seeds studied. His temperature conditions were generally not those of maintained temperatures, though some tests were carried out in a cold-storage room at about 4°. For most of the tests he employed a definite type of fluctuating temperature involving what may be called alternating maintained temperatures; the cultures were transferred twice daily (morning and evening) from one incubator to another, two incubators (each with its own maintained temperature) being employed for any given test. The pairs of maintained temperatures employed were 2° and 10°, 10° and 18°, 18° and 27°, and 27° and 35°. Thus, for example, a culture was subjected to a maintained temperature of 18° during the day time, the temperature was then quickly changed to 10° and the culture was subjected to this lower maintained temperature overnight, the temperature being again quickly changed back to 18° on the following morning, etc. KORSTIAN's results are presented in graphs constructed similarly to those of BOERKER.

Although the present study was conducted along lines somewhat like those followed in KORSTIAN's work, the two plans were essentially different in many fundamental respects, as will appear later. Only maintained temperatures were employed in the present work, excepting as temperature fluctuation was necessitated by the short observation periods (when the cultures were outside of the incubators) and the background conditions were arranged so as to be reproducible with unusually close approximation. The problem, as set up in planning these studies, is much less complex than has generally been the case in earlier studies on the germinative energy of lots of seed and the environmental complexes for which the results are to be considered as valid are here perhaps more closely defined than has thus far been customary in this kind of experimentation.

The main features of this study of conifer seeds may be summarized as follows: The general or background conditions were considered with some care and a sort of standard technique was worked out for them. Light was excluded from the cultures excepting as they had to be removed from the culture chambers for observation. Many different maintained tempera-

tures were employed and many different lengths of the time period of incubation for each maintained temperature. To ascertain the percentages of germination for different environmental complexes and for different initial physiological or internal complexes (lots and kinds of seed) was the immediate aim of the experimentation. The work was planned primarily to advance our knowledge of principles or generalizations that may express the causal relations of maintained temperature and its duration to the percentage of germination occurring in the lots of seed tested; that is, to the germinative energy of each lot as manifested under the conditions of the several tests.

The study here reported was carried out betewen October, 1927, and March, 1928, in the Laboratory of Plant Physiology of the Johns Hopkins University, where the author occupied a position as student assistant. The work was made possible by a furlough granted the writer by the United States Department of Agriculture, which allowed him to be absent from his post with the Appalachian Forest Experiment Station during the academic years of 1926–27 and 1927–28. For himself as well as for the Johns Hopkins Laboratory of Plant Physiology, he wishes to mention with appreciative thanks the kindness of those who supplied the seeds used in the work. Their names are recorded on a subsequent page. For useful information about molds that were encountered in the cultures cordial thanks are expressed to Doctor Carl Hartley, who was once a student in this laboratory, to Mr. L. W. R. Jackson, both of the United States Bureau of Plant Industry, and to Doctor Charles Thom, of the United States Bureau of Chemistry and Soils. The author wishes to express his thanks to Doctor W. H. Tisdale, of the Jackson Laboratory of the E. I. du Pont de Nemours Company, for supplying samples of the antiseptic "Semesan" and communicating valuable information about its composition and use. Finally, he desires to acknowledge his indebtedness to Doctor Burton E. Livingston, director of the Laboratory of Plant Physiology of the Johns Hopkins University, for constant aid and criticism in the planning and conduct of the experiments, in the logical analysis of the results and in their presentation. He is also greatly indebted to his wife, Bessie Amerman Haasis, for helpful suggestion and criticism throughout the course of the work.

Experimental methods and materials

Standard cultures and their treatment

The cultures of this study may be conveniently grouped in two categories, (1) the standard cultures and (2) a number of preliminary or supplemental additional cultures of other types. The standard cultures were all prepared and treated in the same general manner and they and their

treatment will be described first, after which the additional cultures and their treatment will be briefly described, largely as modifications of the standard type.

The seeds were germinated on agar plates in covered glass Petri dishes 9 cm. in diameter and 1 cm. deep, which had been held at a temperature of 140°–160° C. for at least an hour in an electric oven. Sufficient granulated agar ("Bacto-Agar," from the Digestive Ferments Co., Detroit, Michigan) for the entire investigation was thoroughly mixed before work was begun, to form a single stock of this material. Very close similarity in the many lots of agar solution that were to be prepared during the course of the experimentation was thus insured. A stock of "Semesan" (from the E. I. du Pont de Nemours Co., Wilmington, Delaware, described as containing 35 per cent., by weight, of hydroxy-mercuri-chloro-phenol, the balance being about one-third $CaCO_3$ and two-thirds Na_2CO_3) was prepared by mixing in the same way. The water used was from a "Barnstead" still.

The agar plates were prepared as in bacteriological procedure. The granulated agar was poured into the water while the latter was boiling and the mixture was stirred till all agar particles had disappeared. A gram of dry agar was used for each hundred cubic centimeters of water. Different lots of this mixture were made from time to time, for the several series of plates. Twenty cubic centimeters of the liquid agar mixture was used for each standard plate, being pipetted into the Petri dish while hot. Each dish was then immediately covered and allowed to cool.

The seeds, which had been previously soaked in antiseptic solution, were distributed uniformly on the surface of the agar plate, one hundred seeds to a plate. The seeds were arranged in a square pattern of ten rows, ten in a row, at distances of 6 mm. each way. Uniform spacing, secured by using a guide diagram or pattern on paper lying under the dish while the seeds were being distributed, obviated the necessity for tedious counting. The soaked seeds were placed by means of forceps, which were rinsed in standard "Semesan" solution from time to time while the work of distributing the seeds was in progress. The seeds quickly adhered to the agar gel sufficiently to allow the cultures to be freely handled and soon inverted without alteration in the arrangement pattern.

Because preliminary tests indicated that the seeds in an experiment were apt to become moldy before germination occurred, they were regularly soaked, for an hour just before being distributed on the plates, in a solution of "Semesan." This preliminary treatment was found to retard the development of mold. It should be noted that the antiseptic treatment introduces special considerations and may complicate to some extent the analysis of the experimental conditions. The treatment may possibly have resulted

in some cultures giving a somewhat lower percentage of germination than they would have given if the seeds had been soaked in distilled water, but the evidence at hand indicates that such chemical influence was never significant. Without the antiseptic treatment the influence of mold would have been very serious with some of the sets of test conditions.

The standard "Semesan" solution used for preliminary soaking was prepared with one gram of the powder for each 400 cc. of water. Since the proprietary product is stated to contain 35 per cent. by weight, of hydroxy-mercuri-chloro-phenol, this standard solution is taken to contain 0.0875 gm. of this compound in each hundred cubic centimeters. Fresh lots of solution were prepared at intervals as required and the solution was never used when more than five days old.

Most of the seeds tested failed to sink in the antiseptic solution and a special technique was consequently required for the preliminary soaking. Each batch of seeds was soaked in a large-mouth, cork-stoppered bottle, of 250 cc. capacity, nearly filled with solution so as to contain only about 1 cc. of air. During the soaking period the bottles were continuously rotated about a horizontal axis (three or four turns a minute), being so fixed that the long axis of each bottle remained inclined about ten degrees from the axis of rotation. By the rotating of the bottle and the quick movement back and forth of the air bubble adjacent to the seeds, these were kept in constant motion during the period of soaking. Each seed was thus continually bathed in solution and the adjacent solution was continually renewed.

About a thousand seeds were soaked for each series of cultures. At the end of the soaking period the wet seeds were spread out on the plane bottom of a glass dish and were then transferred, one by one, to the agar plates. Only apparently healthy seeds were used, but no attempt was made to select them for size, color, etc., the chief aim being to obtain similar groups of one hundred seeds each, so that all cultures of the same lot of seed might be comparable. Seven cultures were usually included in each series, one for each of the seven different maintained temperatures that were available.

The time required for the distribution of the seeds in a series of cultures was generally less than an hour, being shorter as experience and practice increased. Toward the end of the study the seeds for a series of seven cultures were generally placed on the plates in half an hour. Soaking and placing both occurred at room temperature, generally between about 19° and about 22° C. On account of evaporation, the seeds were probably a little cooler than the air when placed on the plates. It is thus seen that each experiment started by soaking all seeds in standard "Semesan" solution for from 1.5 to 2 hours, with temperatures around 20°. As soon as

the preparation of a series of cultures was completed the cultures were distributed in the maintained-temperature chambers, so that the differences between the temperatures began to be effective about 1.5–2 hours after the seeds were put to soak. Since germination of the seeds studied is relatively slow at temperatures around 20°, it may be supposed that the pre-germination processes had not progressed far during the time period devoted to soaking and distribution. The general consistency of the results obtained indicates that the latter would not have been notably different if the preliminary period had been shorter or if it had been characterized by lower temperatures or by smaller temperature fluctuations.

The maintained temperatures

The battery of controlled-temperature chambers used in this study was described in its main essentials by Livingston and Fawcett (**13**) and its operation was referred to by Fawcett (**6**), for whose studies on the temperature relations of some parasitic fungi it was originally constructed. A number of investigations have been carried out with the aid of this series of chambers in the last twelve years. The apparatus was overhauled in the fall of 1926 and mechanical refrigeration was introduced at that time, to replace ice cooling, which had been used before. The ice-water tank is now filled with calcium chloride brine (about equal parts by weight of the commercial salt and water) and this solution is cooled by a mechanical refrigeration unit such as is commonly employed in home refrigerators. The form used has an electric motor that intermittently operates a piston compressor, sulphur dioxide is the refrigerant and the apparatus is cooled by flowing water.

The introduction of mechanical refrigeration has rendered more convenient the employment of temperatures below that obtained with melting ice, but the mechanical unit used has not been without occasional lapses and need for expert attention and it has perhaps not been, on the whole, more satisfactory than was the daily addition of ice, especially when it has been required that the temperature of the cooling tank should approach room temperature. The features requiring attention (not always at convenient times in an experiment series) have been the motor itself and the thermostatic switch. Only once, at the time of installation, has any notable escape of sulphur dioxide occurred and that accident might have done no harm had the apparatus been located elsewhere than in a greenhouse full of living plants, for of course no cultures were in the chambers at the time. It is suggested that a refrigeration apparatus of the absorption type, without motor or pump, may possibly prove in many cases to be more suitable for use in scientific experimentation than those forms that depend on

mechanical devices for compressing the refrigerant. The series of chambers is located in one of the greenhouse rooms of the laboratory because of the lack of other more suitable space and the air temperature of the room fluctuates considerably. A basement room, preferably with automatic temperature control to within a few degrees of fluctuation, would be preferable to the present location.

The seven culture chambers of this series are cylindrical (38 cm. in diameter and 38 cm. deep) and each opens above. They are arranged in a row, each being provided with its own water-jacket, which has a motor-driven stirrer. Supported from the top of each chamber is another cylinder, of thin, multi-perforated galvanized sheet iron, 28 cm. in diameter and 43 cm. high, with its floor about 2 cm. above the floor of the outer cylinder. The 5-cm. layer of air between the outer and inner cylinders is kept in motion by two vertical stirrer rods, each of which is bent upon itself to form an inverted U, the terminal portion of which operates to stir the water of the jacket. The water-jackets of adjacent chambers are separated by sheet-iron partitions, which prevent any movement of water from jacket to jacket but allow heat transfer by conduction. At each end of the series is a control tank with stirrers and each of these tanks is separated from the jacket of the adjacent chamber by a sheet-iron partition. One of the tanks (at the hot end of the series) contains water, heated by means of an electric heater with thermostatic control. The control tank at the other (cold) end of the series, which also has stirrers, contains calcium-chloride brine and has thus far been operated without heating apparatus. The sheet-iron partition between it and the cooling tank (where refrigeration is applied) has perforations which, in these studies, have been only partially closed by rubber stoppers, so that circulation of brine might occur between these two tanks. The thermostatic regulation of the temperature of the control tank at the cold end has thus been left to the refrigeration apparatus. The water of the chamber jackets and that of the hot control tank is covered by a thin layer of engine oil, to retard evaporation. Sides, bottom and ends of the series as a whole are insulated with a layer of tightly packed oxhair (such as is used in plaster for the interior of buidings) and the top, including the removable covers of the several chambers, is insulated by pine wood and about 3 cm. of sheet cork. The whole is covered above, in addition, by pads of saddler's hair-felt 3 cm. thick, two thicknesses of ordinary wool bed-blankets and a white canvas tarpaulin. In spite of this extra insulation for the tops of the chambers, it appears that most of the heat leakage occurs through this region. A considerable amount of heat leakage is of course inevitably due to the opening of the chambers for observations. Visible radiation is completely excluded from the chambers, and the cultures were therefore in darkness excepting when observation was necessary.

For any set of seven maintained temperatures (or of narrow temperature ranges, to be more precise) it is necessary only to adjust the two thermostats that control the supply and the removal of heat at the ends of the series. A day or two of idle operation follows each new setting, to allow the chambers to approach dynamic equilibrium with one another and with the control tanks, after which each one maintains its temperature indefinitely, generally with a fluctuation of less than a single centigrade degree above and below the mean. The temperature fluctuations of the cultures were of course greater in most instances, on account of their being removed at intervals for observation, but the periods of departure from the mean on this account were kept as short as possible.

The difference between the temperature of the water or brine in either control tank and the air temperature in the adjacent chamber was about 10°, being somewhat larger or smaller according to the settings of the thermostatic controls. The difference between the air temperatures of any two adjacent chambers was of course determined by the two control temperatures in any setting. It was not uniform throughout the series, being usually smaller toward the colder end.

Each chamber is provided with a calibrated thermometer of the ordinary chemical type, which may be momentarily raised and read without disturbing the chamber cover. Temperatures were observed and recorded at frequent intervals in each period of operation and small Richard thermographs were operated in some of the chambers, while maximum-minimum registering thermometers of the Six type were used in some cases. From the available temperature records a mean was derived as accurately as possible for each chamber for each period of operation, to represent the maintained temperature of that chamber for the period in question. The means for eleven periods of operation, considered as the respective maintained temperatures dealt with, are shown in table I, where the eleven periods are numbered chronologically and the seven chambers are numbered serially, beginning at the cold end of the series.

It will be seen from the data of table I that the same chamber was used, at different times, for different maintained temperatures, and that the same maintained temperature was in many instances obtained in different chambers at different times. No relation was discovered between the seed responses and the particular positions (in the greenhouse) of the chambers in which they occurred, and the use of different chambers for the same maintained temperature need not be considered further. Similarly, no relation was observed between the seed responses and the periods of operation in which they occurred; hence the temperature means for all periods of operation of the control chambers may be considered in a single series,

TABLE I

PERIODS OF OPERATION OF MAINTAINED-TEMPERATURE CHAMBERS AND MEAN TEMPERATURES OF THE SEVEN CHAMBERS FOR EACH PERIOD

Period of operation		Temperature means						
Period No.	Dates (1927–28)	1st Chamber	2nd Chamber	3rd Chamber	4th Chamber	5th Chamber	6th Chamber	7th Chamber
		Deg. C.	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*
1	Oct. 14–26	20	25	31	35	42	48	55
2	Oct. 31–Nov. 16	16	20	24	27	33	37	41
3	Nov. 16–18	18	21	26	29	34	37	42
4	Nov. 25–Dec. 8	26	29	33	36	41	45	51
5	Dec. 16–24	8	12	15	18	22	24	28
6	Dec. 29–Jan. 8	20	25	31	36	43	50	57
7	Jan. 11–24	20	24	28	32	36	41	46
8	Jan. 28–Feb. 4	23	26	30	35	39	44	50
9	Jan. 30–Feb. 11	26	28	31	36	40	45	50
10	Feb. 25–Mar. 9	18	22	26	30	36	39	45
11	Mar. 3–27	18	22	26	30	36	40	46

as though all tests had been made simultaneously. The following list, compiled from table I, shows the maintained temperatures actually employed, together with notations as to the corresponding periods and chambers. The period numbers, with subscripts to indicate chamber numbers, are shown in parentheses for each temperature.

8° (5_1)	33° (2_5, 4_3)
12° (5_2)	34° (3_5)
15° (5_3)	35° (1_4, 8_4)
16° (2_1)	36° (4_4, 6_4, 7_5, 9_4, 10_5, 11_5)
18° (3_1, 5_4, 10_1, 11_1)	37° (2_6, 3_6)
20° (1_1, 2_2, 6_1, 7_1)	39° (8_5, 10_6)
21° (3_2)	40° (9_5, 11_6)
22° (5_5, 10_2, 11_2)	41° (2_7, 4_5, 7_6)
23° (8_1)	42° (1_5, 3_7)
24° (2_3, 5_6, 7_2)	43° (6_5)
25° (1_2, 6_2)	44° (8_6)
26° (4_1, 3_3, 8_2, 9_1, 10_3, 11_3)	45° (4_6, 9_6, 10_7)
27° (2_4)	46° (7_7, 11_7)
28° (5_7, 7_3, 9_2)	48° (1_6)
29° (3_4, 4_2)	50° (6_6, 8_7, 9_7)
30° (8_3, 10_4, 11_4)	51° (4_7)
31° (1_3, 6_3, 9_3)	55° (1_7)
32° (7_4)	57° (6_7)

No special attempt was made to control the air composition in the culture chambers nor in the culture dishes themselves. The air over the agar plate must have been generally nearly saturated with water vapor and the germination data indicate clearly that the supply of oxygen (or the disposal of carbon dioxide) was in no case a limiting condition. It should be noted that the covers of the culture chambers and those of the dishes were surely not tight enough to seriously retard gas diffusion and that the air of the chambers generally approached water saturation.

Special cultures and their treatment

Besides the standard cultures, which were used in most of the tests, a number of preliminary and supplementary tests with modifications of the standard culture methods were employed. In some of these less than 100 seeds were used for each culture. In some instances dry seeds were placed on the plates without preliminary soaking, or they were soaked in tap-water or in distilled water instead of "Semesan" solution, or the concentration of "Semesan" and the period of soaking were different from that noted above, or some other antiseptic was employed.

For these special cultures the temperature chambers of the series described above were employed in some instances, and electric incubators were

used in other instances. The latter maintained mean temperatures of 25°, 30°, and 35°, with fluctuation of about one degree above and below the mean. A few cultures were transferred from the regular temperature chambers to incubators before observations were completed. Further details concerning these additional cultures will be presented farther on, as required in the presentation of the results.

Observations, records, computations and graphs

Cultures were usually placed in the chambers in the forenoon, within an hour of the placing of the soaked seeds upon the agar plates, and observations were usually made 21 hours later and at 24-hour intervals thereafter. No effort was made to keep the observation intervals exactly uniform for all cultures of the same series and there was some variation in their length. Cultures were usually prepared and placed in the chambers on several successive days after the beginning of each period of operation. The several series of any period consequently overlapped in regard to time.

Observations were made on small groups of cultures (not more than six), and the rest remained in their chambers till work with the group in hand had been completed and they had been returned. The chambers remained closed excepting momentarily, when cultures were removed or replaced. Observations were made in the shade in the greenhouse, the work being performed as rapidly as possible. All cultures were consequently exposed to conditions other than those of the culture chambers for a short time whenever observations were made. As much as an hour and three quarters might elapse between the observation of cultures from the first chamber and the observation of cultures from the seventh chamber. Observation regularly began with the coldest chamber and proceeded along the series of chambers to the hottest one.

Observation intervals were not always the same for different series. Intervals of a few hours were used in a number of experiments. In a few cases different series were started at 2-hour intervals, so that observations made at about the same time might give data for intervals of several different lengths; *e.g.*, for 10, 12, and 14 hours from the beginning of the test. For experiments in which the observations of the first day or two were to occur at short intervals, the number of cultures was so restricted that the examination of all of them might be completed in fifteen minutes. It is obvious that the number, frequency, and distribution of the observations made on a given culture in an experiment period must have largely determined the number and distribution of the pronounced temperature and other environmental fluctuations to which that culture was inevitably subjected on account of its temporary removal from the chamber for observation.

The Petri dish remained uncovered for a few minutes during each examination of each culture and all germinated seeds were removed with forceps and counted at each examination, the points of the forceps being frequently dipped in standard antiseptic solution. A satisfactory background was afforded by a piece of black paper placed beneath the culture dish during the examination.

The attainment of germination was considered as having occurred as soon as a definite and clearly discernible projection of the embryo had pushed out from the ruptured seed coat. For the conifer seeds here considered this implies the protrusion of the primary root. This is an easily observed stage or phase of the germination process, arbitrarily chosen for convenience. A similar stage was employed by KORSTIAN (10) in his study of germinating acorns. If it seems desirable the attainment of this protrusion stage of germination may be considered as marking with a considerable degree of precision the transition from germinating seed to seedling, but there is not as yet any generally-followed convention in the use of these vague terms. Perhaps many students of seed germination would consider that germination begins with the onset of swelling and that the process continues till leaves appear. However that may be, in the present account the word germination implies simply the attainment of the protrusion stage.

Since observation of any culture could not be continuous and since undesirable environmental fluctuations became more pronounced with more frequent observations, many seeds had progressed far beyond the critical protrusion stage before germination was noted. At the end of a 24-hour period seeds of pitch pine, for example, might exhibit primary roots as much as 5 mm. in length. The inaccuracies thus introduced always tend to render the recorded incubation period for a seed, or the recorded mean period for a number of seeds, too long; it is never too short. Account was taken of these sources of inaccuracy wherever possible. They generally vanish or become insignificant for our present purposes, through the averaging processes to which all numerical data were subjected.

The number of seeds that had germinated in each culture since the last observation was ascertained and suitably recorded for each observation, and the total number that had germinated since the beginning of the test was then obtained for each incubation period, by summation. For example, a certain standard culture incubated at a mean temperature of 32°, which had shown no seeds germinated at the end of the first day, showed 10 germinated at the end of the second day, 30 more at the end of the third day, and 19 more at the end of the fourth day. The percentages of germination for the four different incubation periods were therefore as follows:

Length of incubation period	1 day	2 days	3 days	4 days
Percentage of germination	0	10	40	59

Each standard series thus gave a set of seven values or indices of germinative energy for each incubation period used, one value for each maintained temperature. These indices were plotted to give a temperature-germination graph, with abscissas representing maintained temperatures and ordinates representing the percentage values, and each length of incubation period naturally had its own graph. From a system of such graphs it was easy to observe what was the temperature giving the highest percentage for each length of incubation period, what was the general form of each graph and how the graph form altered from one length of period to another.

When the experimentation had been discontinued all comparable data for each lot of seeds were assembled in a single tabulation and all indices of germinative energy were averaged for each of the several tested maintained temperatures and for each length of incubation period. For example, there were ten values for lot 1 of pitch pine seed for the 3-day incubation period and for the maintained temperature of 41°. These were obtained from three periods of operation of the temperature chambers, as follows: (1) three values from as many cultures in the fifth chamber, (2) two values from two cultures in the sixth chamber, and (3) five values from five cultures in the seventh chamber. The ten comparable values (which ranged in magnitude from 12 to 34) were averaged to give the final average index for this lot of seed for the 3-day incubation period and for the maintained temperature of 41°. The final average index is 20 in this case. An average index was obtained in this manner for each of the remaining temperatures that had been employed for the 3-day period and these were plotted to form a graph for that length of period and for this particular lot of seed. Other lengths of incubation period for the same lot of seed were treated in the same general way, each length of period having its own graph, and these graphs were assembled on one sheet, with the abscissas the same for all.

Seeds used

The lots of seed with which this paper is concerned are listed below.

Pitch pine (*Pinus rigida* Mill.) :—

Lot 1. Crop of 1926, New Jersey. (Received in June, 1927.[a])

Lot 2. Crop of 1926, New Jersey. (Received in December, 1927.[a])

Lot 3. Crop of 1927, New England. (Received in January, 1928.[a])

Lot 4. Crop of 1927, New Jersey. (Received in January, 1928.[a])

Lot 5. Crop of 1927, Franklin Co., Pennsylvania. (Received in February, 1928.[b])

Red pine (*P. resinosa* Ait.). Crop of 1927. (Received in December, 1927.[a])

Scotch pine (*P. sylvestris* L.). Crop of 1927. (Received in January, 1928.[a])

Lodgepole pine (*P. murrayana* Balf.). Crop of 1927. (Received in January, 1928.[a])

Long leaf pine (*P. palustris* Mill.). Crop of 1927. (Received in November, 1927.[a])

Western yellow pine (*P. ponderosa* Laws.). Crop of 1927, Hat Creek Forest, British Columbia. (Received in February, 1928.[b])

Loblolly pine (*P. taeda* L.). Crop of 1927, Pender Co., North Carolina. (Received in February, 1928.[b])

White spruce (*Picea canadensis* BSP). Crop of 1926. New England. (Received in June, 1927.[a])

Engelmann spruce (*P. engelmanni* Engelm.). Crop of 1926, Mt. Ida, British Columbia. (Received in January, 1928.[b])

Smooth cypress (*Cupressus glabra* Sudw.). Crop of 1927. (Received in November, 1927.[a])

Results and discussion

Pitch pine seed, lot 1, standard cultures

Data from standard cultures of lot 1 of pitch pine seed are assembled in table II, where each line is devoted to a single maintained temperature and each column is restricted to a single length of the incubation period. The number of cultures on which each mean germination percentage is based is shown as a subscript numeral following the mean value. These data are shown also by the nine narrow-line graphs of figure 1, there being a graph for each length of incubation period shown in the table. On these graphs abscissas represent temperature means, and ordinates represent percentages of germination. All the graphs are plotted on the same scale but with different base lines, the positions of the several different base lines being shown at the left of the figure ($0_{6-7\text{ hr.}}$ to $0_{14\text{ da.}}$). The temperature scale is shown at the bottom.

Examination of the graphs of figure 1 will make apparent the main contributions of the present paper. While the data on which the graphs are based are not as numerous and extensive as might be wished, yet they are generally consistent and their indications seem to be clear.

The maximal temperature was not reached; even incubation periods as short as 6 or 7 hours showed some seeds to have germinated with the highest

[a] Purchased from C. B. Fleu, Jr., Philadelphia.

[b] Lots marked b were kindly furnished as follows: Pitch pine, lot 5, by Mr. J. S. Illick, Harrisburg, Pa. Western yellow pine, by Mr. A. C. Thrupp, Kamloops, B. C. Loblolly pine, by Dr. C. F. Korstian, U. S. Forest Service. Engelmann spruce, by Mr. P. M. Barr, Victoria, B. C.

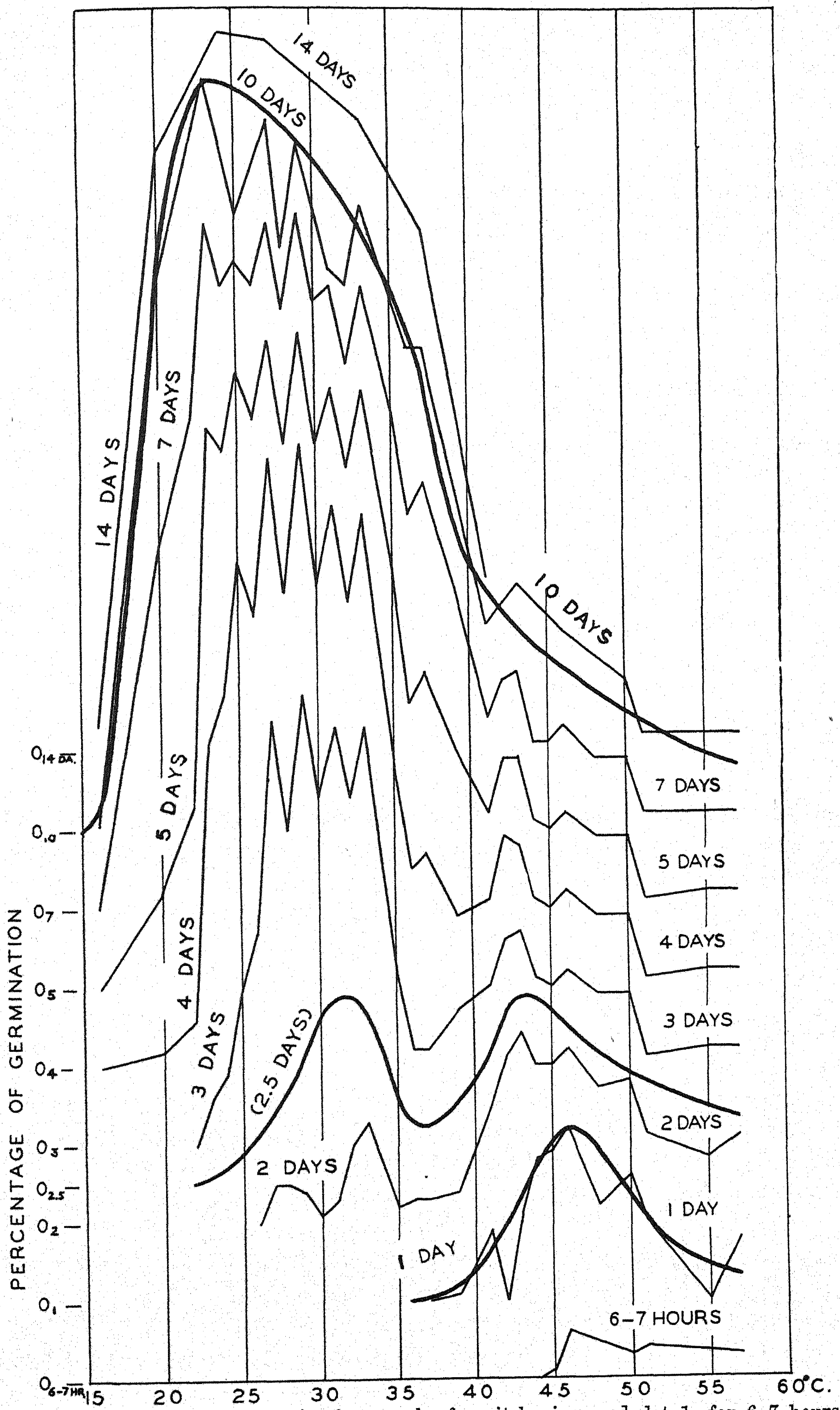

FIG. 1. Temperature-germination graphs for pitch pine seed, lot 1, for 6–7 hours, and for 1, 2, 3, 4, 5, 7, 10 and 14 days of incubation. The broad lines are smoothed graphs for the 1-day and 10-day periods and an interpolated graph for a period of incu-

TABLE II

MEAN PERCENTAGE OF GERMINATION (GERMINATIVE-ENERGY INDICES) OF SEEDS OF PITCH PINE, LOT 1, IN STANDARD CULTURE, FOR MAINTAINED TEMPERATURES BETWEEN 16° AND 57° AND FOR INCUBATION PERIODS OF FROM 6 OR 7 HOURS TO 14 DAYS. (THE NUMBER OF CULTURES ON WHICH EACH MEAN IS BASED IS SHOWN BY A SUBSCRIPT NUMERAL)

MAINTAINED TEMP.	PERCENTAGE OF SEEDS GERMINATED AT END OF								
Deg. C.	6-7-HR. PERIOD	1-DAY PERIOD	2-DAY PERIOD	3-DAY PERIOD	4-DAY PERIOD	5-DAY PERIOD	7-DAY PERIOD	10-DAY PERIOD	14-DAY PERIOD
16	—	0_{5}	0_{5}	0_{5}	0_{5}	0_{5}	0_{5}	1_{4}	3_{2}
20	0_{3}	0_{10}	0_{10}	0_{10}	2_{10}	12_{10}	46_{10}	70_{6}	76_{2}
22	—	0_{2}	0_{2}	0_{2}	6_{2}	23_{2}	62_{2}	—	—
23	0_{1}	0_{4}	0_{4}	6_{4}	41_{4}	71_{4}	87_{4}	95_{2}	—
24	0_{2}	0_{6}	0_{6}	9_{6}	47_{6}	68_{6}	79_{6}	87_{3}	91_{2}
25	0_{1}	0_{6}	0_{6}	20_{3}	64_{3}	78_{3}	82_{3}	78_{1}	—
26	0_{1}	0_{2}	0_{2}	27_{2}	57_{2}	72_{2}	79_{2}	—	—
27	—	0_{5}	5_{5}	54_{5}	77_{5}	82_{5}	87_{5}	90_{4}	90_{2}
28	0_{2}	0_{7}	5_{7}	40_{4}	60_{4}	69_{4}	76_{4}	74_{1}	—
29	—	0_{2}	4_{2}	57_{2}	79_{2}	83_{2}	88_{2}	87_{1}	—
30	0_{1}	0_{1}	1_{1}	44_{1}	61_{1}	69_{1}	77_{1}	—	—
31	0_{1}	0_{3}	3_{3}	53_{3}	71_{3}	76_{3}	79_{3}	71_{1}	—
32	0_{2}	0_{2}	10_{2}	44_{2}	59_{2}	66_{2}	69_{2}	69_{1}	—
33	—	0_{10}	13_{10}	53_{7}	70_{7}	76_{7}	79_{7}	79_{5}	80_{2}
35	—	0_{2}	2_{2}	22_{2}	38_{2}	53_{2}	63_{2}	—	—
36	0_{3}	0_{9}	3_{9}	12_{6}	25_{6}	36_{6}	50_{6}	61_{3}	—
37	—	0_{5}	3_{5}	12_{5}	27_{5}	40_{5}	54_{5}	61_{4}	66_{2}
39	0_{1}	1_{1}	4_{1}	17_{1}	19_{1}	30_{1}	41_{1}	—	—
41	0_{4}	9_{15}	15_{13}	20_{10}	21_{10}	22_{10}	24_{10}	26_{5}	22_{1}
42	—	0_{1}	21_{1}	26_{1}	29_{1}	29_{1}	29_{1}	—	—
43	0_{1}	11_{1}	24_{1}	27_{1}	28_{1}	29_{1}	30_{1}	31_{1}	—
44	0_{1}	18_{1}	20_{1}	21_{1}	21_{1}	21_{1}	21_{1}	—	—
45	1_{2}	19_{8}	20_{6}	20_{3}	20_{3}	20_{3}	21_{3}	—	—
46	6_{2}	22_{2}	22_{2}	22_{2}	22_{2}	22_{2}	23_{2}	25_{1}	—
48	—	12_{1}	17_{1}	19_{1}	19_{1}	19_{1}	19_{1}	—	—
50	3_{2}	16_{2}	18_{2}	19_{2}	19_{2}	19_{2}	19_{2}	19_{1}	—
51	4_{2}	10_{8}	11_{6}	11_{3}	11_{3}	11_{3}	12_{3}	12_{1}	—
55	—	0_{1}	8_{1}	12_{1}	12_{1}	12_{1}	12_{1}	—	—
57	3_{1}	8_{1}	11_{1}	12_{1}	12_{1}	12_{1}	12_{1}	12_{1}	—

maintained temperature employed (57°). For this lot of seed the absolute maximum temperature for germination was therefore surely considerably above 57°.

The minimal temperature for germination is shown to have been about 44° for the shortest period. For more prolonged incubation the minimum is shown to be progressively lower; about 38° for the 1-day period, about 26° for the 2-day period, and below 15° for the 14-day period. With still longer incubation the minimum would surely have appeared as well below, perhaps far below, 15°. To establish the absolute minimal temperature for the occurrence of germination in this lot of seed and for the background con-

ditions of these tests it would have been necessary to continue incubation much longer than fourteen days.

These observations on the temperature minimum emphasize at once the great importance of the duration factor in the consideration of this cardinal temperature. With seeds that germinate slowly it might be necessary to continue an experiment for many years before the absolute minimal temperature for germination could be ascertained. What percentage of the seeds of a lot can germinate at the absolute minimal temperature is another question, for answering which very prolonged incubation of the cultures would also be necessary.

Such considerations as these have been appreciated in a general way by those who have discussed minimal and maximal temperatures for physiological, and indeed for chemical and physical, processes, and it has frequently been noted that these two cardinal temperature values are usually capable of being ascertained only approximately. It may be remarked, however, that the maximum may always be stated as *above* a certain temperature (the highest temperature at which any germination occurred, for example), while the minimum may always be stated as *below* a certain temperature (the lowest at which the process referred to was observed).

With regard to optimal temperatures the graphs of figure 1 are especially interesting. For the shortest of the periods the optimum was only a degree or so above the corresponding minimum, being about 46° (6 per cent.). It was still about 46° (22 per cent.) for the 1-day period, but was somewhat lower, between 42° and 46° (21–24 per cent.), for the 2-day period. A second, and lower, optimum is clearly shown, however, for this same 2-day period of incubation, about 32°–33° (10–13 per cent.), and an intermediate range of maintained temperatures between the two optima (from about 35° to about 39°) gave remarkably low percentages of germination (2–4 per cent.). For the 3-day and 4-day incubation periods the high-temperature optimum (26–27 and 28–29 per cent., respectively) was about 42°–43°, but it had nearly disappeared for the 4-day period and is not evident for the longer periods represented in figure 1. The reason for the disappearance of the high-temperature optimum is clearly due to the fact that relatively few additional seeds germinated at about 42°–43° after the end of the 3-day period, while larger numbers of additional seeds germinated at lower temperatures. The depression in the graph between the two optimal temperatures is progressively less pronounced for the 3-day and 4-day periods, and it fails to appear in the graphs for the 5-day and longer periods of incubation.

The second or low-temperature optimum (32°–33°), shown as the smaller of the two in regard to percentage of germination for the 2-day period, is

represented by the highest percentage values on the graphs for the 3-day and longer periods. For the 5-day and longer periods, moreover, it is the only optimum and the graphs for these periods resemble, in form, many published graphs for the maintained-temperature relations of physiological processes.

For the 3-day period the low-temperature optimum (the one that appears as the only optimum for the longer periods, as just noted) is an optimal range, from about 27° to about 33° (40–57 per cent.); for the 4-day period this range is from about 25° to about 33° (57–79 per cent.); and for the 5-day and 7-day periods the optimal range (66–83 and 69–88 per cent.) is about the same as for the 4-day period. For the 10-day and 14-day periods the optimal temperature range appears to be narrower and located somewhat lower on the thermometer scale; for the longest incubation period represented, this range is from about 24° (91 per cent.) to about 27° (90 per cent.).

From these observations on the optimal temperatures it is very evident that the length of the period of incubation is a very important variable in the determination of the magnitude of the optimum and the characteristics of its range. The specification of this duration factor is even more essential in this connection than it is with regard to the temperature maximum and temperature minimum. As was emphasized by LEHENBAUER (**11**) and by FAWCETT (**6**), the magnitude of the temperature optimum for a process may, in general, be expected to vary considerably with the time factor, and statements as to temperature optima must usually be only vague and relatively unsatisfactory unless the length of the period of exposure is also given.

The importance of the length of the incubation period in the discussion of the characteristics of the temperature optimum for the occurrence of germination in this lot of seed is especially well shown when we consider the remarkable appearance of the double optimum for the 2-day and 3-day periods. It is evident that if these germination tests had been made with only 1-day, or with only 5-day or longer incubation periods, or if the periods used had been of several lengths but in every case less than 1 day or more than 4 days, no double temperature optimum would have been apparent from the numerical results and their graphs.

The three smoothed, broad-line graphs of figure 1 remain to be mentioned. The lowest of these is simply the smoothed graph for the 1-day period and the highest is the smoothed graph for the 10-day period. These give generalized pictures of the forms of the temperature-germination graphs for the short period (when the high-temperature optimum appears alone) and for the long period (when the low-temperature optimum is

alone evident). As the eye moves upward over the figure a continuous transition of the graph form, with progressively longer periods of incubation, is clearly indicated by the intervening narrow-line graphs, which represent the results of actual experiment. We may say that the lowest broad-line graph becomes gradually transformed as the incubation period is longer and longer, until it finally takes the form of the highest broad-line graph. In this continuous transformation the graph must pass through an intermediate form having two maxima with equal ordinates, each graph maximum representing one of the two temperature optima for germination. That particular stage in the graph transformation here envisaged is repre-

Length of period	Maximum	High-temperature optimum (large values)	Intermediate temperature (small values)	Low-temperature optimum (large values)	Minimum
6–7 hr.	57° +	46° (6 p. c.)	———	———	44°
1 da.	57° +	46° (22 p. c.)	———	———	38°
2 da.	57° +	42°–46° (21–24 p. c.)	35°–39° (2–4 p. c.)	32°–33° (10–13 p. c.)	26°
2.5 da.	**57° +**	**43.5° (24 p. c.)**	**36°–37° (7 p. c.)**	**31.5° (24 p. c.)**	....
3 da.	57° +	42°–43° (26–27 p. c.)	36°–37° (12 p. c.)	27°–33° (40–57 p. c.)	22°
4 da.	57° +	42°–43° (28–29 p. c.)	39° (19 p. c.)	25°–33° (57–79 p. c.)	16°
5 da.	57° +	———	———	23°–33° (66–83 p. c.)	16°
7 da.	57° +	———	———	23°–33° (69–88 p. c.)	16°
10 da.	57° +	———	———	23°–29° (74–95 p. c.)	15°
14 da.	57° +	———	———	24°–27° (90–91 p. c.)	15°–

sented by the intermediate broad-line graph of the figure. This is not a smoothed graph for any series of experimental data actually available; it is a hypothetical graph, interpolated between the experimental graph for the 2-day period and that for the 3-day period, and it represents, as its parenthetical label indicates, an incubation period of approximately 2.5 days.

If the period of incubation represented by this interpolated graph had been employed in testing this lot of pitch pine seed the results would have been very striking indeed. For maintained temperatures about 31.5° and about 43.5° the percentage of germination would have been about 24, while it would have been only about 7 for a maintained temperature of about 36.5°.

To summarize the transition in graph form from that for the 6–7-hour period to that for the 14-day period, the cardinal features of the several graphs of figure 1 are tabulated, on p. 391. The interpolated data (for a 2.5-day period) are shown in black-face type.

The maximal temperature is above 57°. The minimal temperature recedes from about 44° (6–7-hr. period) to below 15° (14-day period). The high-temperature optimum recedes from about 46° (6–7-hr. period) to about 42° (4-day period) and then vanishes. The low-temperature optimum recedes from about 32° (2-day period) to about 25° (14-day period). The intermediate temperature, characterized by small percentage values, lies between 35° and 39°, being evident only for periods of from 2 to 4 days.

Special experiments with pitch pine seed, lot 1

Several special experiments with pitch pine seed of lot 1 were carried out with preliminary soaking in distilled water instead of in "Semesan" solution, standard procedure being otherwise employed. The results of these are shown by the graphs of figure 2, which show temperature optima for about 32° and about 41°, for incubation periods of 2 and 3 days, respectively. For the 10-day period a single optimum is shown as the temperature range from below 20° to about 32° (with germination percentages of

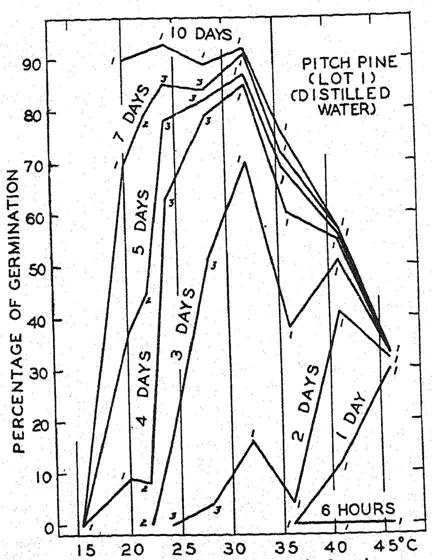

Fig. 2. Temperature-germination graphs for pitch pine seed, lot 1, preliminarily soaked in distilled water instead of "Semesan" solution, for 1, 2, 3, 4, 5, 7 and 10 days of incubation. No germination in 6-hour period. Numerals adjacent to points each indicate number of tests on which corresponding percentage value is based.

from 89 to 93). The temperature maximum is shown as at least above 46° and the minimum appears as about 36° for the 1-day period, about 24° for the 2-day period, about 22° for the 3-day period, and about 15° for the 4-day, 5-day, and 7-day periods. The numerals near the points on the graphs indicate how many cultures were used in each case.

A special experiment in which pitch pine seed of lot 1 was used without preliminary soaking at all, the dry seeds being distributed on the agar plates while the other details of the procedure were standard, showed for a 3-day incubation period one optimum about 30° (42 per cent.) and another for the temperature range from about 40° to about 46° (34 per cent.).

The double temperature optimum exhibited by the standard cultures of lot 1 of pitch pine seed is therefore not to be considered as related specially to the standard preliminary soaking in the antiseptic solution.

It seems clear that the recession of the minimal temperature with prolonged incubation and also the appearance of two separate optimal temperatures with intermediate lengths of the incubation period are related to an innate variability in the lot of seed. Apparently a sample of lot 1 of pitch pine consisted of several groups or categories of seeds, separable by experimentation of the type used in these studies. Turning again to the graphs of figure 1, it is evident that some of the seeds of each sample were capable of germination with an incubation period of 6 or 7 hours while some seeds failed to germinate at any temperature even with a 14-day period of incubation. If we regard the several cultures, of one hundred seeds each, as representative samples of the entire lot, about 20 per cent. of the lot were apparently capable of germination in a single day at a maintained temperature of 45°–46°, while none were able to germinate in a period of this length at any maintained temperature below 37°. With prolonged incubation (10–14 days) only about 30 per cent. of the seeds could germinate at the high-temperature optimum (42°–46°), but at the low-temperature optimum (24°–25°) 90 per cent. of them germinated in the longest incubation period. Most of the seeds characterized by capacity to germinate at the higher temperatures were also characterized by capacity to germinate *quickly* at those temperatures, but these same seeds were apparently capable of germination at the low-temperature optimum if incubation was sufficiently prolonged.

The seeds of this lot 1 may be tentatively considered as roughly grouped in three physiological categories according to their germinative capacity in regard to maintained temperature, as shown for the 14-day period of incubation: (A) those capable of germination at about 25° but not capable of germination at about 43°, (A′) those capable of germination at about 43° as well as at about 25° and (B) those not shown as viable at any maintained

temperature. The categories are not sharply distinguished but it appears that about 65 per cent. of this lot of seed belonged to the first category (A), about 25 per cent. to the second category (A′) and about 10 per cent. to the third category (B). With the third of these hypothetical groups we need not be concerned in the present connection, but the first two groups (A and A′) may be studied somewhat more closely by means of some available experimental data.

Many seeds that had germinated at 50° remained alive but grew only very slowly for as long as 10 days when kept at that temperature. If seeds that had germinated at about 50° were transferred, shortly after germination, to a temperature about 31° none of the seedlings grew well at the lower temperature and some were apparently dead at the time of transfer. Seeds that had germinated at about 43° and had then been immediately transferred to 31° showed seedling growth for 10 days, but the seedlings were apparently unhealthy. On the other hand, seedlings that had been produced at 31° and had then been transferred to 57° were dead within 8 days. In these special tests, the only seedlings that were growing well after 8 or 10 days were those that had been produced at about 31° and had been kept at that temperature.

These results indicate that, although about 25 per cent. (group A′) of the seeds of this lot of pitch pine were capable of germination at the high-temperature optimum, the resulting seedlings failed to develop satisfactorily with such a high temperature and were, indeed, likely to grow very poorly even at lower temperatures.

Other transfers were made from one maintained temperature to another to gain evidence as to whether the higher temperatures had killed the seeds that had failed to germinate quickly; that is, whether seeds of category A were killed by the high temperatures that produced the prompt germination of those of category A′. Cultures that had been exposed for 2 days or more to a temperature of 45° or 51° (in which, respectively, about 20 per cent. or about 10 per cent. of the seeds had germinated at the higher temperature) failed to show any additional germination in a 7-day period after they had been transferred to a temperature of 25° or 30°. In a 100-seed culture transferred from 45°, of which 20 per cent. of the seeds had germinated in 25 hours, 12 additional seeds had germinated at the end of the following 7-day period at 25°. In a culture transferred from 51°, of which 10 per cent. of the seeds had germinated in 25 hours, only one additional seed germinated in the subsequent 7-day period at 25°. Of seeds that had been first incubated at a temperature of 41° for 7 or 9 days (in which time about 15 per cent. or about 20 per cent. of them had germinated), only one or two more germinated at 25° in the next seven days.

Cultures that had been incubated at 36° for 7 days (with germination of about 50 per cent.) or for 10 days (with a somewhat greater percentage of germination) were transferred to 25°, after which many additional seeds germinated, the total percentage of germination 7 days after the change being 80 or 85, about as great as was shown by corresponding cultures transferred from a temperature of 33° to one of 25°. Cultures initially incubated at 37° for from 11 to 15 days showed a slight acceleration in the rate at which additional germination occurred after they had been transferred to a temperature of 25°.

Cultures initially incubated for 7 or 10 days at 25°, 29° or 33° continued to show additional germination after being transferred to 25°. Cultures initially incubated at 16° for 15 days, with a germination percentage of only about 5, showed total percentages of 75 or 95, 3 days after transfer to 25° or 30°.

From the results of these special experiments it appears that when pitch pine seeds of this lot, in standard cultures, were incubated at a high maintained temperature (about 45°–50°) for more than about 2 days, those that had not already germinated in that time at the high temperature had lost their capacity for germination, even at a temperature of about 25°. On the other hand, when seeds that had failed to germinate in 15 days at a low temperature (16°) were transferred to a temperature of 25° or 30°, germination was very prompt and general, and the total percentage of germination at the end of an additional 7 days was about 90. It is therefore apparent that the seeds of category A (capable of germination at 25° but not at 43°) were generally killed by incubation at the higher temperature for a period long enough to produce germination, at that temperature, of the other viable seeds of the same lot (category A′). Seeds capable of germination about the high-temperature optimum germinated there very quickly but produced apparently unhealthy seedlings. These seedlings died if kept at the temperature that produced them and they developed only slowly when transferred to the low-temperature optimum shortly after they had germinated at the high-temperature optimum.

It is of course conceivable that seeds capable of germinating at the higher optimal temperatures might be able to germinate at even higher temperatures and perhaps even more quickly. If that were true a triple optimal temperature for the occurrence of germination might appear. In the present study no temperatures above 57° were tested.

Other lots of pitch pine seed

Graphs for the results given by standard cultures of lots 2, 3, 4 and 5 of pitch pine seed are shown in figures 3 to 6, which are plotted in a manner similar to that adopted for the graphs of figures 1 and 2. The number

of tests (cultures) upon which each percentage value is based is shown in each case by a numeral adjacent to the corresponding point on the graph. The numbers are small in all cases and these experimental results are therefore less well established than are those for the pitch pine seed of lot 1.

For lot 2 (fig. 3) the percentage values are markedly lower than the

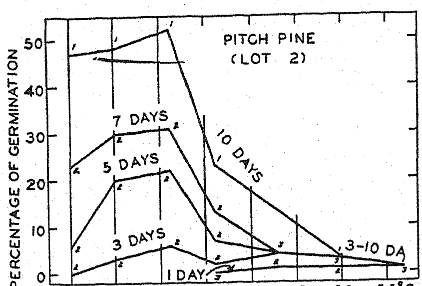

FIG. 3. Temperature-germination graphs for pitch pine seed, lot 2, for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

corresponding indices for lot 1, for all of the maintained temperatures tested and for all the different lengths of incubation period for which data are available. A high-temperature optimum is indicated for the 1-day period and a double optimum (about 31° and about 43°) appears for the 3-day period. The maximum is shown as about 57°. The minimum is perhaps about 36° for the 1-day period and about 20° for the 3-day period. The low-temperature optimum is the range from about 25° to about 31° for the 5-day and for the 7-day periods for which it is the only optimum shown. For the 10-day period this range appears as from about 20° to about 31°.

For lot 3 (fig. 4) no indication of a double optimum appears. The

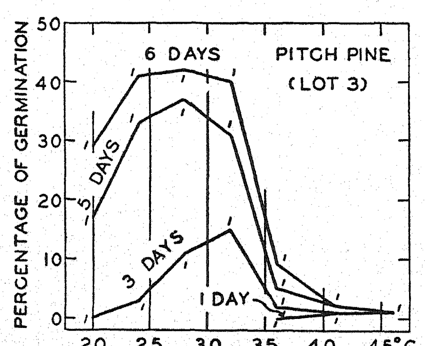

FIG. 4. Temperature-germination graphs for pitch pine seed, lot 3, for 1, 3, 5 and 6 days of incubation. Each percentage is based on but a single culture.

maximum temperature is indicated as above 45°. The minimum is about 36° for the 1-day period and about 20° for the 3-day period. The single (low-temperature) optimum is shown as about 32° for the 3-day period and it appears to be the range from about 24° to about 32° for the 5-day and 6-day periods.

For lot 4 (fig. 5) a high-temperature optimum is indicated for the 1-day

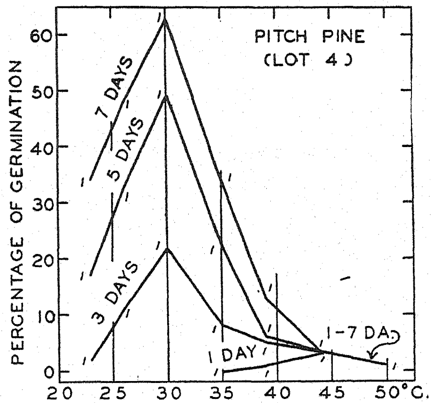

FIG. 5. Temperature-germination graphs for pitch pine seed, lot 4, for 1, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

period (about 44°) and a low-temperature optimum appears about 30° for the 3-day, 5-day and 7-day periods, but no double optimum is actually shown. The maximum appears as above 50° and the minimum is shown about 35° for the 1-day period and below 23° for the 3-day period.

For lot 5 (fig. 6) a high-temperature optimum about 45° or above is

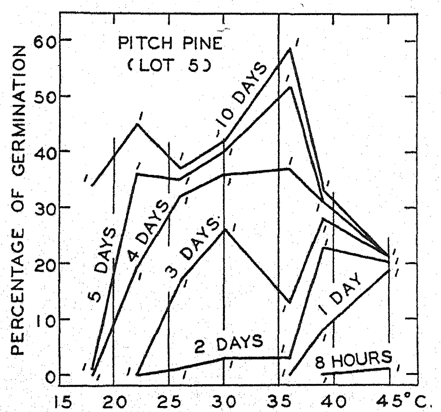

FIG. 6. Temperature-germination graphs for pitch pine seed, lot 5, for 8 hours and for 1, 2, 3, 4, 5 and 10 days of incubation. Each percentage is based on but a single culture.

suggested for incubation periods of 8 hours and 1 day and a similar optimum about 39° is shown for periods of 2 and 3 days. A low-temperature optimum (about 30°) appears with the other for the 3-day period, thus giving a double optimum for that period, with two nearly equal germinative-energy indices. For the longer periods of incubation that were tested the single optimum appears about 36°. The maximum is not indicated (excepting that it is shown as above 45°) and the minimum appears about 39° for the 8-hour period, about 36° for the 1-day period, about 22° for the 2-day and 3-day periods, about 18° for the 4-day period and below 18° for the 5-day and 10-day periods.

In general, the results of these standard tests with lots 2, 3, 4 and 5 of pitch pine seed indicate that lot 1 was peculiar in several respects, but they show that the double optimum so clearly established for seed of that lot may be expected from tests of other lots of this species, though exceptions are to be expected also. It appears that this phenomenon of a double optimum is related to differences (variability) in the internal conditions or physiological characteristics of the seeds of each lot for which it was exhibited. These differences and their distribution in the lot must have acted together with the environmental background conditions of the standard treatment. It does not seem desirable to attempt here to make suggestions as to precise and detailed reasons for the differences between these lots of pitch pine seed, although a number of apparently plausible hypotheses might be imagined. With the meager data at hand it is best to avoid any attempt in that direction and to say simply that the double optimum was shown for some lots of pitch pine seed in standard cultures for relatively short periods of incubation, while it failed to become evident in similar tests of other lots of seed of the same species. If a little deeper but very general analysis is desired, it seems to be safe to say: (1) that the individual seeds of each lot differed among themselves in their capacity to germinate under the several different sets of experimental conditions used in the tests, each set of conditions embracing, besides the standard background complex, one particular maintained temperature and one length of the incubation period; (2) that the seeds of any lot may be conveniently classified in a number of physiological categories according to their individual capacities for germination under the influence of the several experimental conditional complexes; and (3) that each lot was characterized by its own set of proportions of the seeds belonging to the several categories. The graphs of germinative energy as related to the duration of incubation and to temperature furnish pictures of the ways in which the lots differed in statistical make-up. According to these pictures the several lots might themselves be grouped in lot categories.

SEEDS OF OTHER CONIFEROUS TREES

Results of some less extensive experiments with lots of seed of the other coniferous trees dealt with in this study are summarized by the graphs of figures 7–15. The experimental procedure was standard for all but loblolly pine (fig. 9), longleaf pine (fig. 10), western yellow pine (fig. 12) and one series of Engelmann spruce (fig. 13, broken lines). In the case of loblolly pine only 50 seeds were used per culture and the preliminary soaking was in distilled water. In the case of longleaf pine only 25 seeds were used per culture, the procedure being otherwise standard. For Engelmann spruce, the series represented by broken lines in figure 13 differed from the standard only in that the preliminary soaking was in distilled water. The cultures of western yellow pine were standard excepting that only 50 seeds were used for each.

The experimental data for these nine species are in every case much less extensive than those for pitch pine seed, lot 1, and they are to be regarded as not very significant; but several of these series are clear in their indications concerning the double optimal temperature for germination. The graphs for these series are constructed like those previously presented and do not require special discussion, but the following notes on them are of interest.

For red pine (fig. 7) two temperature optima (about 24° and about 36°)

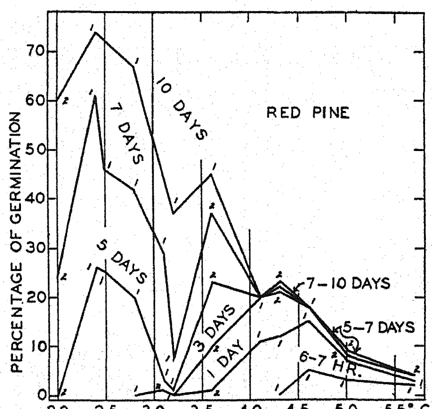

FIG. 7. Temperature-germination graphs for seeds of red pine, for 6–7 hours and for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

are evident for incubation periods of 5, 7 and 10 days. For the two shorter periods tested (6–7 hours and 1 day) only a single optimum is shown (about 46°).

For lodgepole pine (fig. 8) two optima (about 24° and about 40°) appear for incubation periods of 3, 5, 7 and 10 days, and but one optimum

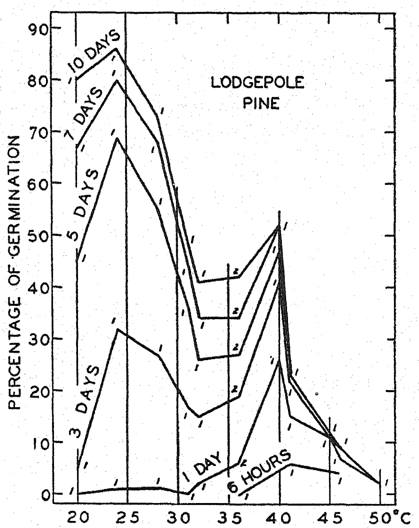

FIG. 8. Temperature-germination graphs for seeds of lodgepole pine, for 6 hours and for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

(about 40°–41°) is indicated for the two shorter periods (6 hours and 1 day).

For loblolly pine (fig. 9) two optima (about 26° and about 40°) appear

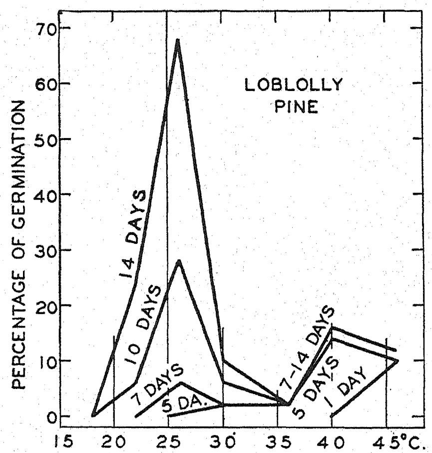

FIG. 9. Temperature-germination graphs for seeds of loblolly pine (50 seeds to the culture and preliminary soaking in distilled water instead of antiseptic solution), for 6.5 hours and for 1, 5, 7, 10 and 14 days of incubation. Each percentage is based on but a single culture.

for 7, 10 and 14 days. A single optimum is indicated for the 1-day period as above 46°, while for the 5-day period it appears to be about 40°.

For longleaf pine (fig. 10) two optima (apparently below 18° and above 46°) are indicated for incubation periods of 5, 7 and 10 days. For periods of 1 day and 3 days a single optimum is indicated as above 46°.

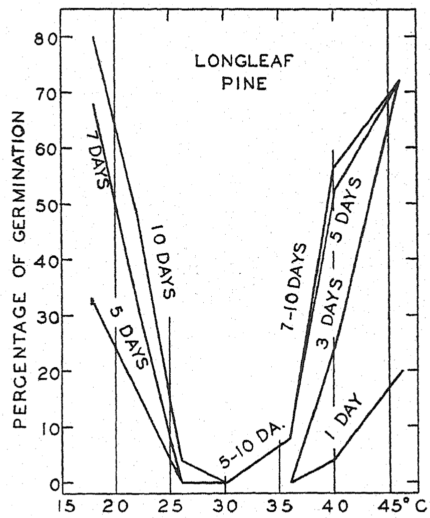

FIG. 10. Temperature-germination graphs for seeds of longleaf pine (25 seeds per culture), for 6 hours and for 1, 3, 5, 7 and 10 days of incubation. Each percentage is based on but a single culture.

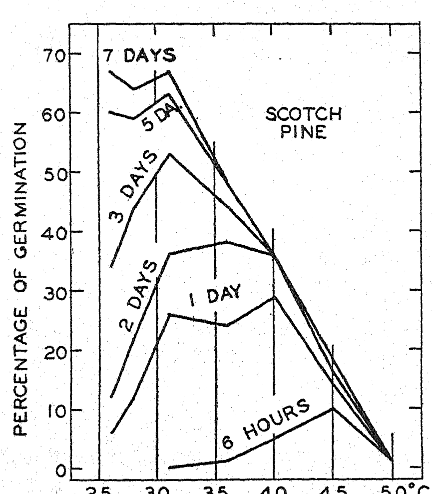

FIG. 11. Temperature-germination graphs for seeds of Scotch pine, for 6 hours and for 1, 2, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

It was not until the tenth day of incubation that any seed germinated with a maintained temperature of 26°, and no germination had yet occurred at 30° when the experiment was discontinued after the cultures had been incubated 10 days. After the seventh day no additional seeds germinated with any temperature above 26°.

For Scotch pine (fig. 11) only a single optimum is indicated; about 45° for the 6-hour period, about 40° for the 1-day period, about 36° for the 2-day period, about 31° for the 3-day and 5-day periods and between 26° and 31° for the 7-day period. Two optima (about 31° and about 40°) are perhaps suggested for the 1-day period. A period of about 18 hours might have shown two optima, if such a period had been tested. It may be noted that SCHIMPER (**20**, page 46) quotes DETMER as stating that the maximal temperature for the germination of seed of Scotch pine is 34°, while the optimal temperature is 27°.

For western yellow pine (fig. 12) only a single optimum is shown;

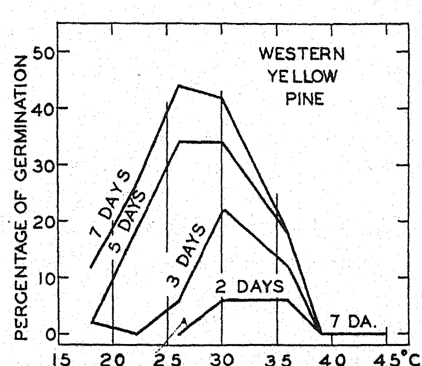

FIG. 12. Temperature-germination graphs for seeds of western yellow pine (50 seeds per culture), for 2, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

between 30° and 36° for the 2-day period, about 30° for the 3-day period and between 26° and 30° for the 5-day and 7-day periods. It is possible that two optima might have appeared for the 2-day period if suitable maintained temperatures between 30° and 36° had been tested.

For Engelmann spruce (fig. 13) there were two series, for one of which the cultures had standard treatment while the seeds used in the other series had been soaked in distilled water instead of the standard antiseptic solution. In figure 13 the graphs for the standard tests are full lines and those for the special tests are broken lines. Two optima are clearly indicated (about 26° and about 40°) for periods of 3 and 5 days, also (about 26° and about 36°) for the 7-day period. A single high-temperature optimum

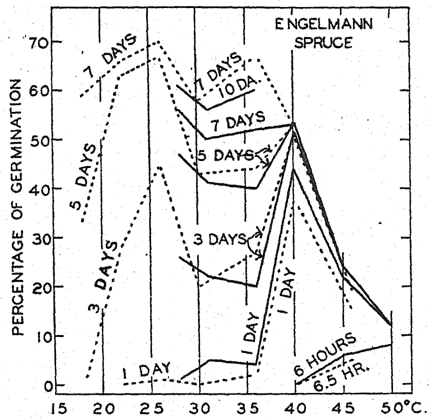

FIG. 13. Temperature-germination graphs for seeds of Engelmann spruce for 6–6.5 hours and for 1, 3, 5, 7 and 10 days of incubation. Solid lines represent standard cultures and broken lines represent cultures of seeds preliminarily soaked in distilled water instead of antiseptic solution. Each percentage is based on but a single culture.

(about 40°) appears for the 1-day period and the same is true (perhaps about 50° or even higher) for the period of 6 or 6.5 hours. As in the case of lot 1 of pitch pine seed, a satisfactory general agreement is shown between the results of the standard cultures and those of the tests with water-soaked seeds.

For white spruce (fig. 14) only a single optimum is indicated; about

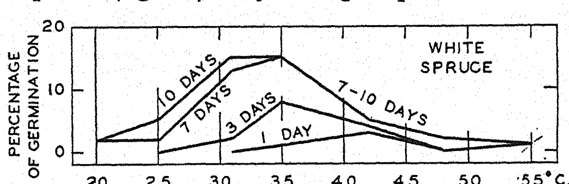

FIG. 14. Temperature-germination graphs for seeds of white spruce, for 1, 3, 7 and 10 days of incubation. Each percentage is based on but a single culture.

42° for the 1-day period, about 35° for the 3-day and 7-day periods, and between 30° and 35° for the 10-day period.

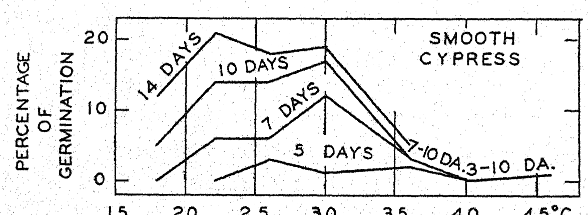

FIG. 15. Temperature-germination graphs for seeds of smooth cypress, for 3, 5, 7, 10 and 14 days of incubation. Each percentage is based on but a single culture.

For smooth cypress (fig. 15) two optima (about 26° and about 36°) are suggested for the 5-day period, but only a single optimum (about 30°) is indicated for the 7-day and 10-day periods, while two optima (about 22° and about 30°) are suggested for the 14-day period.

Résumé of results on temperature optima, and additional notes

For convenience of reference and for comparison the main indications about optimal temperatures for the occurrence of germination in the several lots of seed, as set forth in the preceding sections of this paper and by the graphs of figures 1 to 15, are brought together in table III, the arrangement of which is self-explanatory.

There appears to be no doubt that the double optimum observed for lot 1 of pitch pine seeds is to be expected not only for other lots of seed of that species, but also for lots of seed of five other coniferous trees (red pine, lodgepole pine, loblolly pine, longleaf pine and Engelmann spruce). This expectation is suggested for Scotch pine and smooth cypress also. In some cases the double optimum is indicated for a wide range of lengths of the incubation period while in other cases it is confined to certain intermediate periods of incubation if these are neither too long nor too short.

In connection with the remarkably high maximal temperatures indicated for most of the lots of seed dealt with in this paper (50° or above, and apparently as high as 60° for pitch pine seed of lot 1), it may be remarked that seeds in nature may be temporarily subjected to temperatures above 60° for limited periods of time, as during hot sunny periods, if they lie on or near the soil surface. This may well be of frequent occurrence in the case of some species of pine that grow in areas subjected to high temperature and intense sunshine. For example, BATES (**1**) observed occasional temperatures of 66° C. about 0.5 cm. below the soil surface on south slopes in the central Rocky Mountain region, and TOUMEY and NEETHLING (**23**) record an exreme soil-surface temperature of 67° C. for New Hampshire. When such high soil temperatures occur they are apt to be confined to short periods (perhaps less than 12 hours) but they may be repeated for several days in succession or at longer intervals. It seems doubtful that moisture conditions suitable for seed germination are apt to be present at or near the soil surface when these very high temperatures occur.

In nature germinating seeds are almost always subjected to fluctuating temperature rather than to maintained temperature and the possible ecological bearing of the influence of temperature fluctuations on plant activities can not be approached seriously until much laboratory experimentation and precise analysis have been devoted to the general physiological problem of the influence of fluctuations in temperature as these affect the funda-

TABLE III

SUMMARY OF DATA ON OPTIMAL TEMPERATURES FOR THE OCCURRENCE OF GERMINATION IN THE SEVERAL LOTS OF SEEDS STUDIED, DERIVED FROM TABLE II AND FIGURES 1–14

Kind of seed and treatment	Figure no.	Length of incubation period	Optimal temperature		
			Double		Single
			Low	High	
			Deg. C.	*Deg. C.*	*Deg. C.*
Pitch pine, lot 1 (standard cultures)	1	6–7 hr.	——	——	46
		1 da.	——	——	46
		2 da.	32–33	42–46	——
		3 da.	27–33	42–43	——
		4 da.	25–33	42–43	——
		5–7 da.	——	——	23–33
		10 da.	——	——	23–29
		14 da.	——	——	24–27
Pitch pine, lot 1 (soaked in distilled water)	2	1 da.	——	——	46
		2–3 da.	32	41	——
		4–5 da.	——	——	32
		7 da.	——	——	24–32
		10 da.	——	——	20–32
Pitch pine, lot 2 (standard cultures)	3	3 da.	31	43	——
		5–7 da.	——	——	25–31
		10 da.	——	——	20–31
Pitch pine, lot 3 (standard cultures)	4	3 da.	——	——	32
		5–6 da.	——	——	24–32
Pitch pine, lot 4 (standard cultures)	5	1 da	——	——	44
		3–7-da.	——	——	30
Pitch pine, lot 5 (standard cultures)	6	8 hr.	——	——	45
		1 da.	——	——	45
		2 da.	——	——	39
		3 da.	30	39	——
		4 da.	——	——	30–36
		5–10 da.	——	——	36
Red pine (standard cultures)	7	6–7 hr.	——	——	46
		3 da.	——	——	46
		5-10 da.	24	36	——
Lodgepole pine (standard cultures)	8	6 hr.	——	——	41
		1 da.	——	——	40
		3–10 da.	24	40	——
Loblolly pine* (soaked in distilled water)[a]	9	1 da.	——	——	46 +
		5 da.	——	——	40
		7–14 da.	26	40	——
Longleaf pine* (standard cultures)[b]	10	1–3 da.	——	——	46 +
		5–10 da.	18 –	46 +	——
Scotch pine* (standard cultures)	11	6 hr.	——	——	45
		1 da.	31(?)	40(?)	40(?)
		2 da.	——	——	36
		3–5 da.	——	——	31
		7 da.	——	——	26–31(?)

See next page for footnotes.

TABLE III—*Continued*

Kind of seed and treatment	Figure no.	Length of incubation period	Optimal temperature: Double, Low	Optimal temperature: Double, High	Optimal temperature: Single
			Deg. C.	*Deg. C.*	*Deg. C.*
Western yellow pine (standard cultures)	12	2 da.	——	——	30–36
		3 da.	——	——	30
		5–7 da.	——	——	26–30
Engelmann spruce* (standard cultures)	13	6 hr.	——	——	50 +
		1 da.	——	——	40
		3–7 da.	27 –	40	——
Engelmann spruce* (soaked in distilled water)	13	6.5 hr.	——	——	46 +
		1 da.	——	——	40
		3–5 da.	26	40	——
		7 da.	26	36	——
White spruce (standard cultures)	14	1 da.	——	——	42
		3–7 da.	——	——	35
		10 da.	——	——	31–35
Smooth cypress* (standard cultures)	15	5 da.	26(?)	36(?)	——
		7–10 da.	——	——	30
		14 da.	——	——	22(?)

[a] 50 seeds per culture.

[b] 25 seeds per culture.

* A plus or minus sign after a number indicates that the number is too small or too large, respectively. An interrogation point in parentheses after a number indicates that the magnitude is questionable.

mental plant processes. Such investigation must, in turn, wait for much further advance in our knowledge of the influences exerted on plants by maintained temperature, a subject that has hardly yet begun to receive attention. The whole subject of temperature influence upon organisms constitutes a practically uncultivated field, the problems of which become more complex with each small contribution. Very fundamental physiological research will be needed before these problems may be subjected to sufficiently searching analysis to permit of the planning of experimentation by which they may possibly be simplified and rendered directly approachable.

The phenomena of double temperature optima for the occurrence of seed germination disclosed by the results of the present study are of peculiar interest because of their novelty and they may prove to be of great importance in the study of physiological temperature relations. The possible occurrence of two maintained-temperature optima for germination in a lot of seed is established by the present report, but the relation of these phe-

nomena to plant species and to lots of seed other than those here dealt with, as well as to other influential environmental complexes than those here tested, remains to be taken up. Seed of some species may not exhibit these phenomena at all while that of other species may exhibit them in some lots of seed but not in every lot. The fact that a double temperature optimum for the occurrence of germination has not been discovered for a given kind or lot of seed does not, of course, preclude the possibility that this characteristic may be disclosed in other lots of the same sort of seed or in lots of other sorts. The great significance of the length of the incubation period has been emphasized in earlier sections, and experimentation by which the occurrence of double temperature optima is to be sought must therefore be planned to embrace a number of different lengths of period as well as a number of different maintained temperatures. And the values for both of these variables must be so chosen as to cover the logical field adequately. The lengths of incubation period employed should differ serially by relatively small steps and the same is true of the maintained temperatures used. The range of period length and that of temperature must of course be broad enough to allow the double optimum to become manifest if the lot of seed in question should possess the capacity for exhibiting it. Finally, the environmental background complex employed in such studies as are here suggested (including all influential external conditions excepting temperature and duration) must surely be highly important in the determination of the occurrence or non-occurrence of evidence of a double optimum in any series of tests. It may be surmised that water relations and oxygen relations might furnish limiting conditions in many instances and the mind staggers at the thought of the infinite array of other possibilities suggested by such expressions as chemical influences, and chemical stimulation.

From studies of very different types, double temperature optima have been reported by KOEPPEN (9), for elongation of shoots, and by YAMAHA (**25**), for certain cytological processes in root tips; and the data given by WIESNER (**24**) for rate of germination of fungus spores indicate that he also encountered this phenomenon. The results of these studies cannot, however, be profitably compared with those of the present study.

It may be remarked that the separation of the individuals of a lot of seed into physiological categories, as such a separation or sorting results from studies like the ones here considered, is likely to be related to the general vitality of the seeds, to their varying degree of vital capacity in other respects as well as with regard to viability. It seems probable that the process of respiration may offer useful clues in this connection. If comparative studies on respiration might be conducted concomitantly with thoroughgoing experimentation on germinative energy enlightening results might be expected.

Summary and conclusion

This paper presents the results of experimental studies on the germinative energy of lots of seed, as the measure of this lot characteristic is influenced by the maintained temperature and by the length of the period of incubation employed in the experimental tests. Five lots of seed of pitch pine (*Pinus rigida* Mill.) were studied and several lots of seed of other coniferous trees. The seeds were usually subjected to a preliminary soaking in an antiseptic solution (to retard development of fungi), after which they were regularly distributed on the surfaces of agar plates, each in a glass Petri dish. The cultures thus prepared were themselves distributed among the incubation chambers for each experiment series. Thirty-six different maintained temperatures were employed, ranging from 8° to 57° C. and varying for the most part by intervals of one degree, and temperature fluctuation was usually not more than a single degree above or below the mean for the given chamber. Observations were made at daily intervals or more frequently, the length of the shortest period of incubation being 6–7 hours while that of the longest was 14–15 days. A seed was considered as having germinated when its radicle distinctly protruded from the split seedcoat. The number of seeds that had germinated in each culture was recorded for each incubation period and this number was expressed as a percentage of the total number of seeds in the culture, this percentage value being taken as a numerical index of germinative energy for that sample of seeds for that period and temperature. Mean index values were computed by averaging the corresponding percentage values for all similarly treated cultures. For each lot of seed a series of mean indices was thus obtained for each length of incubation period, there being a single mean index for each of the different maintained temperatures tested for that length of period.

The variables dealt with were: (1) the kind of seed tested (species and lot), (2) the maintained temperature and (3) the length of the incubation period. Temperature-germination graphs present the results, abscissas representing temperatures while ordinates represent the corresponding mean germinative-energy indices. A separate graph is presented for each length of incubation period tested, for each lot of seed studied.

The main aim of these studies was to ascertain approximately for each lot of seed the optimal maintained temperature for the occurrence of germination, for each length of incubation period tested. Some attention was given to the other cardinal temperatures also (minimal and maximal) and to the manner in which the form of the temperature-germination graph alters with the length of the incubation period.

For any germination to occur among the seeds of the lot of pitch pine seed to which this study was mainly devoted, the maintained-temperature maximum appears to have been above 57°, perhaps as high as 60°. The minimal maintained temperature is shown as about 44° for the shortest incubation periods (6 or 7 hours) that gave any germination at all. For progressively longer incubation periods the minimal maintained temperature appears to have been progressively lower and a 14-day period of incubation gave a minimum below 15°.

For the shortest incubation periods (6–7 hours) that gave any germination in this lot of pitch pine seed, the optimal maintained temperature was near the minimum, about 46°. For long periods (4–14 days) the optimal temperature appears as a single optimal range extending from about 23° to about 33°, the upper limit of the range being progressively lower for longer periods. For incubation periods of intermediate length a *double optimum* is clearly and unmistakably shown by the experimental data, a high-temperature optimum (corresponding to the single optimum for the shortest periods) and a low-temperature optimum (corresponding to the single optimum for the longest periods). For a 3-day period of incubation these two optima are represented by the temperature ranges from 27° to 33° and from 42° to 43°, respectively. The temperature-germination graph for this intermediate length of period consequently shows two high regions with a pronounced depression between. If a hypothetical graph for an incubation period of about 2.5 days is constructed by interpolation, the germinative-energy index for the high-temperature optimum (43°–44°) becomes numerically the same (24 per cent.) as that for the low-temperature optimum (32°–33°). This means that, for a properly chosen length of incubation period this lot of seed should be expected to exhibit about the same high percentage of germination with maintained temperatures about 33° and about 43°, while the expected corresponding value for an intermediate temperature about 36°–37° is very much lower (about 7 per cent.).

The phenomenon of a double maintained-temperature optimum (two different optima) for the occurrence of germination in a lot of seed is here reported apparently for the first time. It is clearly shown for lot 1 of pitch pine seed and is indicated for lots 2 and 5, but was not evident for lots 3 and 4. It therefore appears to have characterized some, but not all, of the lots of pitch pine seed studied. Similar pairs of temperature optima are shown for single lots of seed of red pine (*Pinus resinosa* Ait.), lodgepole pine (*Pinus murrayana* Balf.), loblolly pine (*Pinus taeda* L.), longleaf pine (*Pinus palustris* Mill.) and Engelmann spruce (*Picea engelmanni* Engelm.), and they are suggested for Scotch pine (*Pinus sylvestris* L.) and smooth cypress (*Cupressus glabra* Sudw.). A single lot of rice seed (*Oryza*

sativa L.) was somewhat thoroughly studied in connection with the experimentation here reported, without the discovery of any indications of a double temperature optimum. It is possible that double optima may be found to characterize the temperature-germination relations of lots of many kinds of seed.

A lot of seed may be classified in groups or categories, according to the different proportions of its individuals that are capable of germination under the influence of different sets of temperature and duration conditions. For example, lot 1 of pitch pine seed, for which the most extensive data are available from this study, may be considered as having been made up of at least three different physiological forms or types: (A) seeds capable of germination in 14 days or less at a temperature about 25° but not at a temperature about 43°, (A′) seeds capable of germination at both of these maintained temperatures within 14 days or less, and (B) the rest of the lot, not capable of germination in 14 days at any temperature, with the background conditions that were employed. These three categories appear to represent, respectively, about 65, 25 and 10 per cent. of the lot. Seeds of category A were permanently injured when incubated for a day or two at about 43° and they failed to germinate subsequently when transferred to a temperature about 25°. Seeds of category A′ germinated very quickly at about 43° but they produced unhealthy seedlings, which grew but slowly even after being transferred to a temperature about 25°. The high-temperature optimum is thus seen to favor quick germination of the seeds of category A′, but apparently at the expense of subsequent vitality in the seedlings produced.

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OSMOTIC PRESSURE, OSMOTIC VALUE, AND SUCTION TENSION

WILLIAM A. BECK

(WITH TWO FIGURES)

Introduction

The term osmotic pressure has been widely misapplied to various osmotic quantities in plant physiology, particularly before the year 1916.

In that year URSPRUNG (**45, 46, 47, 48, 49**) began a critical examination of the literature referring to osmotic quantities in plant cells, and set about giving precision to the term "osmotic pressure." He has coined some new terms for the osmotic quantities which are entirely different in concept and numerical value from "osmotic pressure." He furthermore devised ways and means of measuring the quantities which he defined.

For several years his reform in terminology has been generally accepted in Europe. The paper which the writer read for URSPRUNG (**44**) before the International Congress of Plant Scientists at Ithaca, New York, in 1926, was well received.

Even at this date the transactions of the Congress have not appeared in print, so that the ideas of URSPRUNG are not sufficiently clear to many in this country. It is to help clarify the thinking in this country that this paper has been written. Another motive was to show the relations and differences between the quantities which are most frequently confused, *i.e.*, osmotic pressure, osmotic value, and suction tension.

URSPRUNG's paper as it will appear in print will not indicate the original sources, since practically all of the studies in the domain of the newly-defined quantities were carried out in his laboratories. A larger bibliography is appended to this work than may seem necessary, in order to help those who wish to take up intensive work in this field.

Osmotic pressure

With keen insight VAN'T HOFF (**20**) perceived a relation between PFEFFER's (**35**) remarkable osmotic experiments with plants and the properties of solutions. This discovery led to his theory of solutions, a theory which has come to be regarded as indispensable in physical chemistry, and in both plant and animal physiology.

As is well known, certain sensitive plants like *Mimosa* respond readily to stimuli. PFEFFER attempted to explain the responses of such plants, and

was able to show that certain cells of the pulvini decrease in volume during response, simultaneously exuding water into the intercellular spaces. After some time, the cells were able to absorb the water from the intercellular spaces again, so that the original volume was once more restored, and the tone was automatically reëstablished.

In an effort to measure the active forces which caused the cells to contract and expand, the contracted tissues were stretched, and the force necessary to cause the stretching was determined. The results showed that the active forces in the cells were very great. There remained no doubt that the pressure on the inner side of the cell wall was at least between two and four atmospheres, when the cell was in normal condition. The nature of the phenomenon seemed to be the same as that observed in the experiments with the osmometer of DUTROCHET, so that it was natural to suppose that the force exerted by the cell under tension was equal to the force which caused the water to enter the cell, and furthermore, that the equilibrant of this force was comparable to the hydrostatic pressure (or weight of the mercury when mercury was employed) in the osmometer. Just as the equilibrant force prevented the solution in the osmometer from taking in more water, so did the pressure exerted by the wall prevent more water from entering the cell.

The order of the figures obtained with the osmometer of DUTROCHET did not warrant the conclusion that the phenomenon could be explained by osmosis alone. By using the semipermeable membranes of TRAUBE the results obtained were more satisfactory, and were of the order expected. Some of the results are given in table I.

TABLE I

THE OSMOTIC PRESSURE OF SUCROSE

CONCENTRATION IN PER CENT.	OSMOTIC PRESSURE IN CM. OF MERCURY	RATIO OF PRESSURE TO PER CENT. CONCENTRATION
1	53.5	53.5
2	101.6	50.8
4	208.2	52.1
6	307.5	51.3

From the first two columns it appears at once that the equilibrant of the force which causes the water to enter, "osmotic pressure," is a function of the concentration. The coefficient expressing the relation of the pressure to the concentration (given in the last column) is particularly interesting, and is fairly constant. It indicates that there must be some relation be-

tween the number of molecules of the solute present and the potential force of water influx, which is measured by the equilibrant.

Other experiments showed that the pressure exhibited by the osmometer is a function of the temperature and the relation may be expressed by the formula:

$$p = n \times 0.652 \times (1 + \alpha t) \qquad (1)$$

in which p is the pressure, n is the number of grams of the solute per 100 grams of solution, t the absolute temperature in centigrade degrees, and α a temperature constant determined experimentally.

This mathematical expression reminds one of the formula expressing the gas law. The analogy becomes more striking when it is noted that the accepted value of α is 0.00367 or 1/273.

VAN 'T HOFF used the results of PFEFFER in an attempt to prove that the above formula is entirely analogous to the gas law, *i.e.*, the constant R which is given by the ratio $pv/273$ is the same in the formula expressing the gas law, and in the one expressing the law of osmotic pressure.

According to the gas law the constant R is give by

$$pv/273 = 0.0821$$

in which p is the pressure, v is the volume of the gas, and 273° is the absolute temperature at 0° centigrade. Particular attention is to be given to the fact that in the gas law the pressure is real and expressed, and the volume is the actual volume, while in the osmotic law the pressure is a potential pressure, and the volume is an ideal volume.

If we consider the volume of a given solution of definite concentration, as the volume which is required to contain a gram molecular equivalent of solute, and the pressure as the equilibrant of the force with which water is forced into the osmometer, we can calculate the value of r, which appears in the mathematical expression of the osmotic law. From PFEFFER's results p was found to be 0.652 for a 1 per cent. solution of sucrose at 0° C.; 100 cc. of normal sucrose solution would contain 34.2 grams. The volume of a 1 per cent. solution required to contain 34.2 grams of solute would be 34.2 times the unit volume (100 cc.). From this it follows that,

$$pv/273 = 0.652 \times 34.2/273 = 0.0817 = r \qquad (2)$$

This example illustrates that the constant r which is used in deducing the osmotic pressure, is practically the same in numerical value, as the constant employed in the gas law.

When a solution is separated from the pure solvent by a semipermeable membrane, and a difference of the pressures (measured directly) exerted on the solution and solvent produces a condition of equilibrium (mechan-

ical), such that there is no tendency for the solvent to flow in either direction through the membrane, the difference in pressure is defined as the "osmotic pressure."

VAN'T HOFF based his discussion of solutions on the data of PFEFFER, which were the best to be had at the time. Since, MORSE and FRAZER (**32, 33**) obtained much more reliable results. MORSE showed that much better harmony and exactness resulted if the volume is expressed in terms of the solvent, rather than in terms of the solution. In table II a comparsion of the calculated results according to the two systems is given.

TABLE II

COMPARISON OF THE CALCULATED OSMOTIC PRESSURES

CONCENTRATION OF SUCROSE		OSMOTIC PRESSURE CALCULATED ACCORDING TO		OBSERVED OSMOTIC PRESSURE
WEIGHT NORMAL (MOLS. PER 1000 GM. OF WATER)	VOLUME NORMAL (MOLS. PER LITER OF SOLUTION)	MORSE AND FRAZER	VAN'T HOFF	
0.1	0.098	2.40	2.36	2.59
0.2	0.192	4.81	4.63	5.06
0.3	0.282	7.21	6.80	7.61
0.4	0.370	9.62	8.90	10.14
0.5	0.453	12.00	10.90	12.75
0.6	0.533	14.40	12.80	15.39
0.7	0.610	16.80	14.70	18.13
0.8	0.685	19.20	16.50	20.91
0.9	0.757	21.60	18.20	23.72
1.0	0.825	24.00	19.80	26.64

It is evident from the figures that some definite law governs the force with which the water enters the osmometer, and there can be no doubt that this force is a function of the absolute temperature and of the concentration of the solution. It is just as evident that the manner of expressing this dependence on the temperature and concentration is important, if a perfect harmony with the gas law is to be expected. It remains to be proven that the simple bombardment of the solute particles will explain the pressure in question in the same way as can be done for a confined gas (*cf.* **28**, pp. 127–131).

VAN'T HOFF was interested mainly in the mathematical side of the relations and not so much in the mechanism or the visualization of the molecular activities. He did not mean to prove that the solute was active and the solvent passive or serving as a mere medium in which the solute molecules might move. This seems clear from his statement: "Before entering on the

proof, which deals with osmotic pressure and semipermeable membranes, *i.e.,* membranes that only allow the solvent to pass, it may be remarked that any notion one may form as to the mechanism producing the osmotic pressure, or the action of semipermeable membranes, is without influence on the reasoning. Thus the question whether the pressure is produced by the solvent or by the dissolved body can be left out of consideration; so too, whether it is dependent on collision or on attractive forces. The action of the membrane too, whether it is as a sieve or by means of absorption, is indifferent. All this is the case because the proof to be given is based on thermodynamics, and is consequently free from assumptions on the mechanism.'' (See **21**, p. 32.)

As the above quotation suggests, some place the cause of the osmotic pressure in the membrane and others in the combined or independent activities of the solvent and the solute.

The kinetic theory supposes that the pressure is the result of the bombardment of the membrane by the solute, or the difference in the forces resulting in the bombardment by the solute and solvent. An experiment by RAMSAY (**38**) is often quoted in current literature to give support to this view: A palladium bulb filled with nitrogen at 280° C. was surrounded by a stream of hydrogen. The pressure in the bulb increased almost as much as the total pressure of the hydrogen. For all that, serious difficulties present themselves when an attempt is made to make the kinetic theory of gases entirely analogous to the kinetic theory of osmotic pressure.

The hydrostatic theory considers the solvent as playing the chief rôle in the activities, and attributes the resulting pressure to the entrance of the water into the confined solution. The cause of the entrance is supposed to be the attraction which the solute has for the solvent. This attraction might result from the differences in the surface tension of the solvent and the solution (**41, 42, 30**).

According to PICKERING (**36, 37**) and ARMSTRONG (**1, 2**) the osmotic pressure results from the hydrodynamic conditions, *i.e.,* the hydraulic pressure exercised by the extra molecules of hydrone attracted into it by the complexes formed by the solute and hydrone.

Some authors do not express the ideas of VAN'T HOFF correctly in connection with his formula (*e.g.,* HENDERSON (**19**, p. 76)), and others have not observed the limitations which he makes for his deductions concerning the osmotic pressures of dilute solutions (in which neither the aggregate volume of the solute molecules, nor their mutual attraction are of moment (**20**, p. 479). When more concentrated solutions are dealt with, it becomes necessary to modify the simple equation for osmotic pressure for ideal solutions, in a manner analogous to the modification of the gas law by VAN DER WAALS.

MORSE (**31**, p. 99) thinks it doubtful if any proposed general equation for osmotic pressure (although containing suitable terms for all the factors which must be taken into account) would be of any present utility in the case of aqueous solutions, since the value of at least some of these must still be determined experimentally for every solute, at every temperature, and in each individual concentration.

Other more complicated equations have been suggested by various authors, but they are unsatisfactory because of the complexity of forms that make it difficult to ascribe any definite meaning to their empirical constants. (*Cf.* **6, 10, 11, 12, 13, 25, 26, 34, 62.**) MORSE held that if a suitable equation is eventually established it will be the fruit of extensive and painstaking experimental research.

From all this it is clear that if any deductions about the osmotic quantities in plant tissues are to be made from the isotonic conditions of the cell sap and a chosen plasmolyzing medium, the medium chosen should be one whose osmotic pressures have been determined experimentally for different concentrations, at various practical temperatures.

MORSE (**31**, p. 184) established the osmotic pressures for cane sugar between 0° and 80° C., the concentrations varying from 0.1 to 1.0 m. BERKELEY and HARTLEY established a reliable table of osmotic pressures for the same medium, the concentrations going beyond 2 mols. (See **5**, p. 486, and **7**, p. 271.)

For reasons which he explains, MORSE (**31**, p. 97) preferred to work with the weight normal system (gram-molecular weight of solute per 1000 grams of solvent) rather than in the volume normal system (gram-molecular weight of solute per 1000 cubic centimeters of solution). The weight normal system however, is not practical for the investigation of the osmotic relations in plants, so that it became necessary to translate the values of the osmotic-pressure equavalents for various concentrations of sugar solutions, from the weight normal system to the volume normal system. This work was done by URSPRUNG and BLUM. (See **48**, and **29**, p. 442.)

The osmotic value

Since the cell sap is a solution of varying concentration, the term osmotic pressure can be employed in the sense defined above when referring to its various concentrations. The pressure which would have to be mechanically impressed to cause water to be exuded, plus the pressure of the cell wall upon the cell sap, would measure the osmotic pressure. Care must be taken not to confound the osmotic pressure of the sap with the actual pressure of the wall upon the cell sap, or its equilibrant, the turgor. This error occurs rather frequently in current literature. (For examples refer to **53**, p. 209.)

MORSE (**31**, p. 99) pointed out that the osmotic pressure of a given solution can be known only when accurate measurements have been made at different concentrations and at different temperatures with a satisfactory osmometer. It is impossible to do this for a cell or a tissue. In lieu of absolute values relative values may serve the purpose.

DE VRIES (**60, 61**) first attempted to establish such relative values for the osmotic pressure of the cells. He employed the mature epidermal cells of *Curcuma ribicaulis, Tradescantia discolor* and *Begonia manicuta.* These cells had colored cell sap, which helped considerably in noting the first stages of plasmolysis. He reasoned that at this point the concentration of the cell sap must be equivalent to the concentration of the plasmolyzing agent. It would not have been legitimate to conclude that the osmotic pressure (as above defined) of the cell sap in the normal condition was the same as the osmotic pressure of the solution producing incipient plasmolysis. DE VRIES was mainly interested in comparing the relative osmotic pressure of the plasmolyzing agents, so that his procedure would have been correct for his purpose, provided the cells had responded in the same manner towards all of the plasmolyzing agents; later investigations proved, however, that such was not the case.

Since there can be doubt about the relative pressure in atmospheres it is more secure, and just as logical, to speak in terms of the relative concentration of the agent employed, and then draw whatever conclusion is legitimate from the data obtained. URSPRUNG and BLUM (**53**) proposed the term *Osmotic Value,* and expressed it in terms of the molal concentration of the plasmolyzing agent. They recognized that the osmotic value varies with conditions, and distinguished three different osmotic values for the cell: (a) The osmotic value at incipient plasmolysis, symbolized as O_g (g for Grenzplasmolyse); (b) the osmotic value of the cell sap in the normal cell, symbolized as O_n; and (c) the osmotic value of the cell sap when the cell is saturated with water, symbolized as O_s.

Some authors seemed to have noticed the difficulty of expressing the osmotic pressure of the cell sap in atmospheres, and consequently expressed the relative concentration of the agent, calling this the osmotic pressure. (See **53**.) This was not logical and caused a great deal of confusion. Pressure is the force per unit area $[ML^{-1}T^{-2}]$ and the concentration is the mass per unit volume $[ML^{-3}]$. It is wrong to cofound the quantities in this way.

While making a study of the relative merits of different plasmolytic agents, the writer found it advantageous to append the formula for the agent to the symbol O_g. Thus $O_g(KNO_3)$ represents the osmotic value of the cell sap at incipient plasmolysis, when the agent potassium nitrate is employed, and $O_g(C_{12}H_{22}O_{11})$ represents the osmotic value of the cell sap

at incipient plasmolysis, when cane sugar is employed as the agent. Brevity and exactness of expression are of the utmost importance in this field of work (**4**).

Isosmotic and osmotic coefficients

In an *a priori* way it might be supposed that equal molal concentrations of all substances should have equal osmotic pressure; *e.g.*, 101 gm. of potassium nitrate should have the same osmotic pressure as 342 gm. of cane sugar when contained in 1000 cc. of solution.

De Vries (**60**) showed that this is not true, but that the potassium nitrate is about 1.5 times as active osmotically as cane sugar. In general he divided the substances into groups according to their osmotic activity, based upon the value of cane sugar. Giving the sugar the value 1, the series expressing the relative osmotic pressures was 1, 1.5, 2, and 2.5. In order to obtain a series of whole numbers he arbitrarily chose the value 2 for cane sugar, and obtained the series 2, 3, 4, and 5. These numbers were called isosmotic coefficients. If, for instance, the osmotic pressure of a potassium nitrate solution were to be deduced from an equimolar sugar solution, the osmotic pressure of the sugar solution would have to be multiplied by 3/2, this being the ratio of their isosmotic coefficients.

The dissociation of certain substances upon dissolving, is the chief cause for their higher osmotic effectiveness, and for this reason the isosmotic coefficients roughly represent the degrees of dissociation of the salts.

Fitting (**16, 17**) tested out the isosmotic coefficients with greater exactness than De Vries, and found that for specific substances the variation from the values 2, 3, 4, and 5 was considerable; consequently there was no good reason for retaining the basic number 2. He preferred to express the coefficients on the basis of 1 for cane sugar, and called them osmotic coefficients. They are symbolized by i. In effect then, the osmotic coefficient of a given substance is the ratio of the osmotic value of a given cell sap at incipient plasmolysis using cane sugar as an agent, to the osmotic value of the same cell sap using the substance in question as an agent. It follows, for instance, that

$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)} \tag{3}$$

expresses the osmotic coefficient for potassium nitrate (**4**, p. 24).

The value 1.69 was the best that Fitting could obtain for the potassium nitrate coefficient, using the low concentrations that plasmolyze the epidermal cells of *Rhoeo discolor*. When he determined i cryoscopically he found the value to be 1.78.

The value of i as determined physiologically for potassium nitrate, is invariably lower than the value which is determined by physico-chemical

methods. This is chiefly due to the penetration of the potassium nitrate into the cell. (See **17**, and **4**, pp. 48, 50, 54, 55, 58 and 63.)

When cells of higher O_g values are employed, the *i* values are considerably lower than that found by FITTING. Reasons for this behavior were given elsewhere (**4**, p. 28).

Certain agents may stimulate the activity of the enzymes in such a way as to increase the O_g values, thus causing considerable variation in *i* from the ideal value. The guard cells are particularly to be suspected for this possible source of error (**4**, pp. 50 and 65).

FITTING showed that the value obtained for *i* in the case of certain substances by the plasmolytic method was not constant, when different kinds of plants were used. Employing *Begonia* leaves he obtained the value 1.0 for glycerol, but when he employed the leaves of *Rhoeo* the value was 0.77. Since glycerol penetrates in many cases, this was at first thought to be a sufficient explanation of the discrepancy, but a closer analysis proved that the basic cause still remains to be found.

Constancy of O_g

When the solute can penetrate into the cell-sap cavity during the immersion of the cell in a given medium, the O_g value may increase with the time of immersion. In such a case the medium must be avoided, or at least the most favorable time of immersion must be determined and employed. (See **4**, pp. 22, 56 and 58.) Cane sugar seems to be the most reliable agent for most cells in this respect. (*Cf.* pp. 53, 56 and 62.)

It might be supposed that the true O_g value of a mature cell should not change, *i.e.*, while the normal cell may take in water or transpire water under the influence of various external factors, the nature and quantity of solute might be supposed to remain constant, and therefore the osmotic value at incipient plasmolysis to remain constant also, since the amount and nature of the solute determine the O_g.

It is, however, not always true that O_g remains constant. The guard cells seem to vary most in their O_g values, when subjected to the influence of various factors. ILJIN (**24**) noted a tremendous increase in O_g values when the plants were placed in water-vapor saturated atmosphere. He used potassium nitrate as an agent, hence an error in measurements might have been suspected. His results were confirmed by STEINBERGER who also used potassium nitrate as an agent. WIGGANS (**63**) also noted an increase using $CaCl_2$ as an agent but it was considerably less than that recorded by ILJIN. The results of URSPRUNG and BLUM (**57**, p. 17) also confirmed the increase in O_g. They used cane-sugar. In a considerable number of experiments which have not yet been published, the writer noted that not all plants show an increase of O_g in the guard cells, when treated in the manner indicated

by ILJIN. Many plants show regular diurnal variation of the O_g of the guard cells.

When a cell can change its O_g value under normal conditions, it must be ascribed to the variation in the nature or the quantity of the solutes in the cell sap or to both. It follows that SCHWENDENER's theory as to the cause of the opening and closing of stomata must be revised. It is not surprising that the O_g of different tissues in the same plant, or even the same organ of a given plant should be different. While the turgor may be the same, the concentration of the cell sap at incipient plasmolysis can be different, since the quantity of water given off by the cell—until the tension in the wall is relieved—need not be the same in all; nor is the capacity of the cells generally the same. From this it follows that the value O_g does not determine the turgor as some writers (**53**, p. 209) erroneously supposed.

According to the ideas of some investigators, a more or less regular increment of O_g might be expected in the tissues of a plant, starting from below and going upward. URSPRUNG and BLUM found that this view is unfounded. In table III are given some of the results they obtained on the stem of *Urtica* (*cf.* **44**).

TABLE III

VALUE OF O_g (KNO_3) IN THE TISSUES OF THE STEM OF *Urtica*

REGION	PITH	HADROME	INNER CORTEX	OUTER CORTEX	EPIDERMIS
Top	0.35	0.47	0.38	0.44	0.39
Middle	0.4	0.62	0.52	0.55	0.43
Base	0.43	0.66	0.53	0.53	0.59

It is interesting to note that the gradient is in the reverse direction, except in the outer cortex where the value 0.55 is discordant. Furthermore it is to be noted that there is no gradient radially but that the value of O_g seems to be characteristic for a given type of tissue, *e.g.*, the outer and inner cortex have almost the same value in each region of the stem.

Since it is not the gradient of O_g which decides the direction in which the water will flow, there is nothing paradoxical in this result; since the walls of the younger cells offer less resistance than the older ones, young cells will take in more water than the older ones before a condition of equilibrium is established. There are several other variables beside O_g that must be taken into consideration before the actual pressure existing in the normal cell can be calculated.

According to HANNIG (**19**) hydrophytes manifest a greater O_g in the leaves than in the roots.

URSPRUNG and BLUM determined that the O_g in the root tips is greater near the tips and decreases regularly in the first 8 mm. from the tip.

The cells of a given tissue do not as a rule have the same O_g value (**4**, p. 56); nevertheless the mean value is, as a rule, characteristic for that tissue. This was shown nicely in a study made by the writer on herbaceous and woody plants. All of these plants grew in the same well-cultivated garden soil. The results are recorded in table IV.

As investigations, the results of which have not as yet been published, proved that some tissues show a considerable diurnal and annual variation in their O_g value, the tests were made at about the same time of the day, and about the same time of the year. An effort was made to have the conditions for each woody plant the same as for a given herbaceous plant. The measurements were made in the early part of the day because the effect of the sun on the O_g of the guard cells is quite different in different plants.

The difference in the mean values for the epidermis and the guard cells in the herbaceous plants is 0.0875 in favor of the guard cells. This is very little. In woody plants it is also negligible, *i.e.*, 0.075. The difference between the values for cells of the epidermis and spongy parenchyma (chosen to represent the assimilating tissue) in the herbaceous plants is 0.2325, which is considerable. So also is the difference considerable between the similar tissues in the woody plants, *i.e.*, 0.261.

Comparing the values, tissue for tissue, it is seen that the cells of woody plants have considerably higher values than those of the herbaceous plants. There is not a single exception, when the data are compared horizontally across the table. The mean value for the epidermis in the woody plants is greater than that for the same tissue in the herbaceous by 0.2025. The mean values for the guard cells differ by 0.19 in favor of the woody plants. In the same order the difference in the mean values for the spongy parenchyma is 0.23. These differences are significant, since, other things being equal, the cells with less elastic walls demand a higher concentration in the sap to draw the same amount of water. It is furthermore significant that the difference 0.225, 0.19 and 0.23 are almost equal.

The osmotic value of a given cell or tissue can vary under the influence of various factors (**3**). Decreasing temperature causes an increase in O_g, and so also does an increase which is sufficient to injure the protoplasm (**3**, p. 54). According to BÄCHER, light seems to increase the O_g value, but the results of investigations by the writer, which will be published later have shown that different tissues need not respond in the same way in this respect. Red light is more effective than green, and blue less effective in increasing the value. The O_g increases also with drought and with decreasing relative humidity of the atmosphere.

TABLE IV

COMPARATIVE O_g ($C_{12}H_{22}O_{11}$) OF THE TISSUES IN LEAVES

Herbaceous plants						Woody plants					
ATE 1925	Time of day	Plant	Lower epidermis	Guard cells	Spongy parenchyma	Date 1925	Time of day	Plant	Lower epidermis	Guard cells	Spongy parenchyma
gust 17	8: 30 A.M.	*Solanum nigrum*	0.425	0.675	0.575	August 14	8: 00 A.M.	*Ligustrum vulgare*	0.65	0.75	0.975
gust 17	6: 30 A.M.	*Crambe maritima*	0.375	0.65	0.65	August 14	6: 30 A.M.	*Hibiscus syriacus*	0.6	0.55	0.8
gust 17	7: 00 A.M.	*Hesperis matronalis*	0.55	0.6	0.725	August 22	6: 30 A.M.	*Fraxinus excelsior*	0.775	0.825	1.1
ust 18	6: 30 A.M.	*Euphorbia lathyrus*	0.25	0.275	0.575	August 21	6: 30 A.M.	*Cytisus laburnum*	0.675	0.9	1.075
ust 18	7: 00 A.M.	*Osmunda regalis*	0.475	0.425	0.875	August 22	7: 00 A.M.	*Robinia pseudacacia*	0.525	0.7	0.9
ust 18	8: 00 A.M.	*Polygonum orientale*	0.375	0.55	0.675	August 21	7: 00 A.M.	*Liriodendron tulipifera*	0.6	0.675	0.825
ust 27	9: 00 A.M.	*Musa sinensis*	0.4	0.35	0.45	August 21	8: 00 A.M.	*Hedera helix*	0.55	0.575	0.675
ust 29	7: 30 A.M.	*Datura stramonium*	0.3	0.425	0.55	August 29	7: 30 A.M.	*Crataegus oxyacantha*	0.55	0.575	0.85
ember 4	7: 30 A.M.	*Acanthus spinosus*	0.475	0.525	0.625	September 4	7: 30 A.M.	*Castanea sativa*	0.575	0.625	0.775
ember 5	8: 30 A.M.	*Dioscorea batatas*	0.35	0.375	0.6	September 5	8: 30 A.M.	*Populus nigra*	0.5	0.575	0.625
		Mean values	0.3975	0.485	0.63			Mean values	0.6	0.675	0.861

According to MEIER (27) the O_g is greater for plants growing in rocky and mountainous regions than for plants growing in the plain.

These examples prove that the nature and quantity of solute in the mature living cell, need not be constant, and that variations in this respect are seemingly to be considered as adaptive responses to the plant's environmental conditions.

Suction tension

The cells, as they occur in the plant, can obtain the necessary water directly from a given source, or from another cell, by virtue of the force which tends to bring the solute and water together. We are not interested here in the nature of this force. It will suffice for our purpose, that such a force exists and that we can express the force numerically per unit area. By experiment it has been shown, that this force which tends to draw or press water into the cell, can be counteracted entirely or in part, by the pressure of the wall against the sap. The influx of water tends to dilute the sap and increase the pressure of the wall; consequently a point is reached at which the influx of water must cease, even though the sap is still fairly concentrated. Just at what instant this point is reached depends on many factors and cannot be decided by the osmotic value alone as many have erroneously supposed.

The cell may be regarded as an entity under tension, capable of absorbing water in proportion to its tension. If the tension is real or not need not be decided for the moment. Just as osmotic pressure is an ideal pressure, which is not expressed directly and mechanically, so also is this tension in the cell an ideal quantity; furthermore, we need not necessarily commit ourselves to the view that water is drawn into the cell rather than forced into it, by using the term, "suction tension of the cell," any more than the acceptation of the expression "osmotic pressure," as above defined, demands that we definitely adhere to the kinetic theory of osmosis. These terms refer to the nature of the experimental data obtained, and not necessarily to the nature of the forces underlying the phenomena.

There are other cases in physics in which quantities are known to exist, even though they do not receive expression in such a manner that we can directly take cognizance of their dimensions, notably the cases involving potential energy. The potential energy is expressed in units having the dimensions of extension, mass, and acceleration; but the mass does not move through a given distance, and there is no acceleration of the mass.

URSPRUNG calls this tension "suction force" and distinguishes between the "suction force of the contents" of the cell and the "suction force of the cell." The term is not well chosen, since it is not meant to express the total force which the sap or the cell exerts, but the force per unit area.

This is the sense in which URSPRUNG and BLUM constantly use the term and they express the quantity in atmospheres and not in the unit of force. It is unfortunate that URSPRUNG and BLUM (53) did not note the error at the time when they so ably began to clear up the confusion which was caused by the misuse of the term "osmotic pressure."

The term "suction force" does not satisfy the dimensions of the quantity which it is intended to express. The dimensional equation of force is:

$$[F] = [MLT^{-2}]$$

and that of pressure or tension is:

$$[P] = [ML^{-1}T^{-2}]$$

URSPRUNG and BLUM (53, p. 202) formulated the law of the "suction force" as follows: "Es ist Saugkraft der Zelle = Saugkraft des Inhaltes – Wanddruck."

The suction tension of the contents of the cell is not identical with the osmotic value of the contents of the cell, but only equivalent. The suction tension of the contents of the cell is given by the equivalent in atmospheres of the molal expression of the osmotic value of the same, which may be symbolized:

$$S_i \asymp O_n \tag{4}$$

The influx of the water causes tension in the wall which acts antagonistically to the suction tension of the contents; hence the difference between the numerical value of the suction tension and the numerical value of the wall pressure is the resultant suction tension of the cell as an entity. The authors have symbolized their law by:

$$S_z = S_i - W \tag{5}$$

in which S_i is the "suction force" (suction tension) of the contents of the cell, W the wall pressure, and S_z the "suction force" (suction tension) of the cell.

This equation brings the error of calling the suction tension "suction force" into relief, since the minuend and the subtrahend must be expressed in the same units, in order that the operation may be logical. Suction tension may be considered a negative pressure, and can be expressed in atmospheres. According to the equation, however, units of force (*e.g.*, pounds) should be subtracted from units of pressure or tension (*e.g.*, atmospheres), and the result should be expressed in units of force, according to the terms, but the authors actually express the results in atmospheres; all of which is quite illogical.

From equation (5), it is evident that if the wall pressure is constant, the suction tension of the cell varies in the same degree as the suction tension of the contents of the cell; *e.g.*, two cells having the same wall pressure at a given instant have the same suction tension when the suction tension of the contents is the same in both cells. With the influx of water, however, the suction tensions of the two cells will probably soon differ, since the capacities of the cells and the coefficients of elasticity of the walls will probably not be the same.

In general the suction tension of the contents decreases with the influx of water, the wall pressure increases, and consequently the suction tension of the cell decreases.

The equation shows clearly that at incipient plasmolysis, the suction tension of the cell is equal to the suction tension of the contents of the cell, since the wall is completely relaxed and its pressure is zero. Cases are on record where the loss of water in certain cells of specialized tissues was so great that the walls were not merely completely relaxed but even folded like a spring, so that they could exert a negative pressure; consequently the suction tension of the cell was greater than the suction tension of the contents of the cell. It is furthermore clear from the equation (5) that when the wall pressure equals the suction tension of the contents of the cell the suction tension of the cell is zero, *i.e.*, the influx of water must cease. The use of this equation presupposes that the cell is excised, and that the source from which the cell is to receive water is pure water. In a series of cells, in which these relative values form a regular gradient, it is reasonable to suppose that the water will flow from cell to cell in the direction of the gradient, but it would be difficult, if not impossible, to express the absolute suction tension which actually exists between any two of the cells as they lie normally in the plant.

Höfler (**22**, p. 290) first tried to show graphically the relation of three quantities expressed in the equation for the various stages, as the cell passes from a condition of incipient plasmolysis to the condition of saturation. His graphs are given in fig. 1. The wall pressure variations are given by the straight line ($G\ O_T$). Ursprung assumed that the tension in the wall increased directly as the volume increased, so that the straight line is determined if the two points G (volume at incipient plasmolysis, with wall pressure zero), for incipient plasmolysis, and O_T (volume determined and pressure determined), for the point of saturation, are known. The curve O, O_t and O_T is given by the variation of the suction tension of the contents of the cell (determined from the respective osmotic values), while passing from the condition of incipient plasmolysis to the point of saturation. The suction tension of the cell is given by the vertical lines (S, S) between the curve and the straight line.

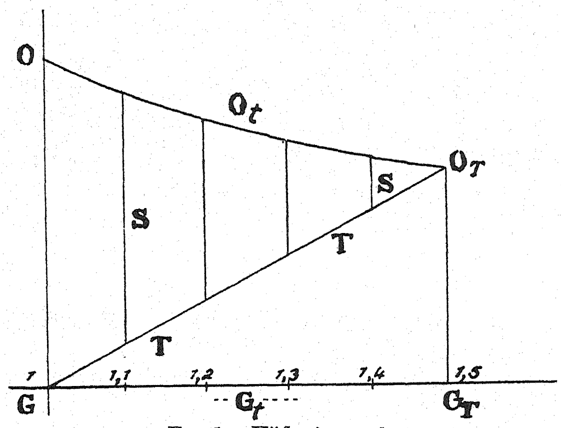

FIG. 1. Höfler's graph.

In fig. 2 are given the relations of the various quantities as they were found in the illustrative example which follows. It is to be noted that the axis of ordinates has been carried to the left of the point where the volume is unity (as given by HÖFLER) in order to avoid confusion. The straight line has been carried beyond the abscissa, to suggest that a cell, in which the cell wall becomes folded, can develop a suction tension greater than the suction tension of the contents of the cell.

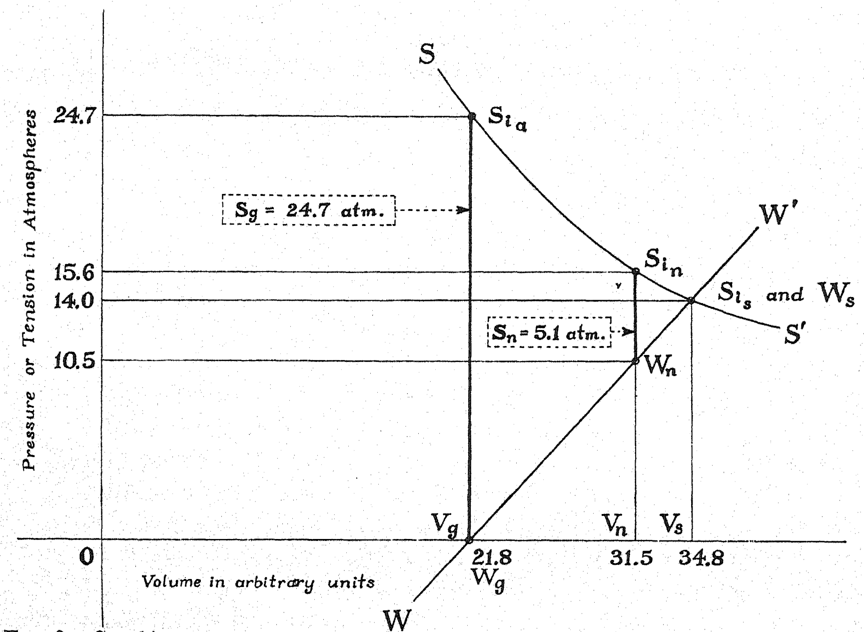

FIG. 2. Graphic representation of the relations of the osmotic quantities in a cell, passing from the condition of incipient plasmolysis to the state of saturation.

The volume of a cell in the normal state was found to be 31.509 (arbitrary units). At incipient plasmolysis the same cell had a volume 21.799, and at the point of saturation 34.779 units. These values were laid off to scale along the abscissa. The osmotic value of the cell sap at incipient plasmolysis $[O_g(C_{12}H_{22}O_{11})]$ was found to be 0.78 mol, which is equivalent to a suction tension of the contents of the cell (S_{i_g}) of 24.7 atmospheres (obtained from the tables of URSPRUNG and BLUM according to the determinations of MORSE).

The osmotic value of the contents of the cell at the point of saturation (O_s) was obtained from O_g as follows: Since the concentration varies inversely as the volume (in the volume normal system), when water is added to a given solution, we may write:

$$\frac{O_s}{O_g} = \frac{V_g}{V_s} \text{ and } O_s = O_g . \frac{V_g}{V_s} \tag{6}$$

Replacing the values O_g, V_g and V_s by 0.78 mol, 21.799 units and 34.779 units respectively, in equation (6), the value 0.49 mol was obtained for O_s. This is equivalent to 13.987 atmospheres (S_{i_s}).

In a similar manner the osmotic value of the contents of the cell in the normal condition was obtained. It was 0.54 mol (O_n) which is equivalent to 15.6 atmospheres (S_{i_n}).

The values for S_i were then plotted along the ordinate against the units of volume, and the three points of the curve were determined. The straight line was determined by the two points V_g (21.8, 0) and S_{i_s} coincident with W_s (34.779, 13.987).

The vertical lines were then dropped from the three known points of the curve for the variation in S_i (S, S′). The vertical for S_{i_n} intersected the straight line representing the variation in volume (W, W′) at the point W_n.

The vertical distances between the curve and the straight line were drawn heavier in order to emphasize the fact that these vertical differences represent the suction tensions of the cell in the different stages. At incipient plasmolysis (S_g) the suction tension was 24.7 atmospheres, in the normal condition (S_n) it was 5.1 atmospheres. The vertical distance from V_n to W_n (representing the wall pressure existing in the normal cell) was 10.5 atmospheres.

The wall pressure of the normal cell could also have been obtained by direct calculation. As was mentioned previously, URSPRUNG assumes that the wall pressure varies directly with the increment in the volume of the cell; therefore we may write:

$$\frac{W_n}{W_s} = \frac{V_n - V_g}{V_s - V_g}, \text{ and } W_n = \frac{V_n - V_g}{V_s - V_g} . W_s \tag{7}$$

When W_n and W_s are the wall pressures, respectively, at normal and saturation points $(V_n - V_g)$ and $(V_s - V_g)$ are the respective increments in the volume. By inserting the known values in equation (7) the value of W_n was found to be 10.5 atmospheres.

Another method of finding the suction tension of cells, in which no account is taken of related osmotic quantities, is based on the principle that a cell which is in dynamic equilibrium with the solution in which it is immersed (will neither increase nor decrease its volume) has a suction tension which is numerically equal to the osmotic pressure of that solution. In practice, that concentration of the given agent in which the immersed cell does not vary its volume must be determined.

URSPRUNG and BLUM have employed this method and compared it with the first and found the results satisfactory. It is simple and yet reliable.

These two methods are particularly adapted to the investigation of the relative values of the suction tensions of the cells in a given tissue, organ, or entire plant.

The associated authors made a painstaking analysis of the suction tensions in the various regions of a plant, under varying conditions. The results were very gratifying and they warrant the expectation that new vistas in plant physiology have been opened, since hitherto unsolved problems have yielded to their methods.

It is evident from the general trend of the literature that a steady gradient of the osmotic value at incipient plasmolysis was thought to exist in the direction of the streaming water (**39, 40, 18, 14**).

To illustrate that the conditions need not be so, the following study of the writer will serve. When the stems of certain succulent plants are cut and left to dry it is noted that the older leaves wilt first and shrivel up, while the younger ones remain turgescent much longer, and in their axes young shoots begin to sprout. PRINGSHEIM claimed that the younger portions had a higher osmotic value at incipient plasmolysis and hence could draw water from the older portions as from a reservoir. To check his claim the stems of *Sedum telephium* were used. The general behavior was as stated above. The result of the mean osmotic values at incipient plasmolysis $[O_g(C_{12}H_{22}O_{11})]$ for different tissues of the older and younger leaves, at different times after the stems were cut, are given in table V. The leaves were numbered beginning at the apex, and proceeding from the younger leaves to the older ones. At each successive test a different leaf was chosen since injury might affect the osmotic value.

It will be noted in the first two tests, which were made immediately after cutting, that the older and younger leaves showed equal values in the respective tissues, except in the lower epidermis, where there was a differ-

TABLE V

$O_g(C_{12}H_{22}O_{11})$ IN OLDER AND YOUNGER LEAVES OF *Sedum telephium*

ORDER OF LEAF	DAYS AFTER CUTTING	$O_g(C_{12}H_{22}O_{11})$ OF THE TISSUES				
		UPPER EPIDERMIS	LOWER EPIDERMIS	GUARD CELLS	PALISADES	SPONGY PARENCHYMA
38	0	0.25	0.225	0.275	0.450	0.450
5	0	0.25	0.200	0.275	0.450	0.450
32	2	0.25	0.225	0.225	0.350	0.350
6	2	0.25	0.250	0.225	0.425	0.350
28	6	0.25	0.225	0.300	0.375	0.375
7	6	0.30	0.275	0.300	0.375	0.375
25	8	0.25	0.225	0.325	0.400	....
8	8	....	0.275	0.350	0.375	....

ence of 0.025 mol in favor of the older leaf. On the second day after cutting, the lower epidermis of the younger leaf had a greater O_g than the equivalent tissue of the older leaf. The difference was 0.025 mol. On the sixth day the younger leaf again showed a higher O_g value in the epidermal tissues but not in the assimilating tissues. On the eighth day the guard cells showed a difference in favor of the younger leaf and again in the lower epidermis, but the palisades showed a higher value in the older leaf.

Evidently the differences in favor of the younger leaves (similar and unsatisfactory results were obtained for the tissues of stems) were never consistently sufficient to support the view of PRINGSHEIM.

If it is recalled that the coefficient of elasticity is less in the young cell walls than in the older ones, and that accordingly the wall pressure (other things equal) is less in the younger cells, it becomes evident that the suction tension in the younger cells is greater; which fact witnesses to the correctness of PRINGSHEIM's basic idea, that the younger tissues obtain their water from the older tissues.

In general, the results of investigations which had as object to prove that the O_g gradient was in the direction of the streaming water, proved to be disappointing. (See **45, 46, 47, 50, 51, 52.**)

On the other hand, a gradient for the suction tensions (S_n) in the direction of the streaming water has been established on several occasions by URSPRUNG, BLUM and HYOZ. From the tips of the roots to the transpiring parenchymatous cells of the leaves, URSPRUNG and BLUM established a regular gradient for S_n, while a similar gradient did not exist in the same plant for O_g. HYOZ (**23**) established the S_n gradient in the leaves of *Hedera helix*. On the cross-section of the stem, URSPRUNG and BLUM found the S_n gradient to run from the water conducting hadrom to the epidermis. In the roots

the conditions were reversed up to the endodermis; here an unexpected and sudden drop was found. They speak of this as the "endodermis jump" (**54, 43, 44**).

This jump is difficult to explain. URSPRUNG and BLUM claim to have proven that a "polar differentiation" exists in the endodermis cells and again in the vascular parenchyma. The accepted S_n value is supposed to be the mean value of a suction tension on one side of the cell, which is considerably greater than the mean, and a suction tension on the other side of the cell which is considerably less than the mean (**58, 44**). This is illustrated in the table in which the values obtained by them on the root of *Vicia faba* are given.

TABLE VI

S_n OF THE TISSUES OF THE ROOT OF *Vicia faba*

TISSUE		S_n	S_n
5th cortical layer			3.2 atm.
6th cortical layer			4.0 atm.
Endodermis	outer side	4.7 atm.	2.6 atm.
	inner side	0.5 atm.	
Pericycle			1.6 atm.
Vascular parenchyma	outer side	4.5 atm.	2.6 atm.
	inner side	0.7 atm.	

URSPRUNG (**58, 44**) outlines his method of measuring the polar differentiation of the suction tension and gives most interesting data, but does not explain how such a strange physical condition, which, apparently at least, contradicts the law of equilibrium, can exist continuously in certain cells. Since the "suction force" law should apply in the particular case, the cause must be sought either in a difference of the molal concentration of the contents of the cell, or in a difference in the tension of the cell wall. It is difficult to see how a considerable difference in the concentration of the contents of the cell should continue to exist for a great length of time (perhaps constantly) and then in a gradient opposite in direction to the influx of the water. It is even more difficult to see how a difference of tension in the cell wall could continue to exist in different parts of the same wall. Even if such a difference could be proven, the final effect of all the different pressures upon the contents of cells should be the mean value of all of the different pressures, and the mean pressure should be equally conducted in all directions within the contents of the cell; thus it is difficult to see how the suction tension of a cell should be different at any two points on a cell.

The method employed admits of the possibility that one and the same cell might manifest different suction tensions at different times (since the

measurements are made successively and not simultaneously and each measurement demands a considerable amount of time). This condition however is not probable, since the lower values were invariably found on the same relative side of the cell.

The measurement of polar differentiation in suction tension is complicated and difficult, but the clearing up of this mystery seems important enough to warrant patient and intense investigation.

If due allowance is made for the faulty nomenclature of the time, it is easy to see that FITTING (**15**) had the correct impression of the osmotic relations in the cell and the real cause of the influx of water into it, when he studied the relations in desert plants in 1911. He interpreted the reason for the high concentration of the cell sap and the low tension in the wall, and said that the "suction force" (Saugkraft) must be very high.

It is interesting to note that in hydatophytes just the contrary condition prevails, *i.e.*, the suction tension is very low, the concentration of the sap being low and the tension in the wall being high.

Mesophytes and hygrophytes take intermediate positions between these two extreme cases (see **29**, p. 500).

Another method for measuring the suction tension which URSPRUNG and BLUM (**56**) devised is particularly well adapted for measuring the mean S_n of tissues, organs or entire plant sections. It is called the simplified method. Whole sections are immersed in the agent, and the concentration at which the solution and section are dynamically balanced is determined. The osmotic pressure of that particular solution is taken to be numerically equal to the mean S_n of the section.

With the aid of this method URSPRUNG and BLUM determined the periodic variations of the suction tensions in response to external factors. It was found that the moisture of the soil exercises the greatest influence on the S_n. The variations depend on the relative resistance which the plant encounters in extracting moisture from the soil rather than on the absolute degree of dryness. This fact suggests (**55**) a practical method of measuring the relative resistances of different kinds of soils to the extraction of water by plants. The relative humidity was found to be the next factor in importance regarding the influence exercised on the suction tensions of the plant.

As might be expected from the effect of the external factors, environment exercises a great influence on the suction tension, which in turn affects the general character of the plant. The suction tension of an organ in a plant remaining in the same location, can vary from 10 to 20 atmospheres in a relatively short time. Different species growing in the same place can manifest widely different suction tensions simultaneously. To judge the

effect of environment, it is best to obtain simultaneous measurements on the same species in different localities. This is not as difficult as it appears to be, since the suction tension of cells and organs remains constant for a long time after being excised, if they are preserved in paraffin oil (**23**). Blum (**8, 9**) made many comparable measurements of suction tension of different species in different localities, and used the values of simultaneous measurements made on *Taraxacum* and *Bellis* growing in those localities as standards. The relation between the suction tension and alpine habitats is illustrated in table VII. The principle that the suction tension increases with the dryness of the soil was demonstrated in the plains as well as in the mountains. The differences in suction tensions are undoubtedly due to the differences in the water balance, which is expressed by the ratio of the transpiration to the absorption; it is symbolized:

$$B = \frac{T}{A} \tag{8}$$

in which T expresses the transpiration and the A the absorption. (See **59**.)

TABLE VII

Suction tension of *Lotus corniculatus* in alpine habitats

Habitat	S_n in atmospheres
Moist sod	9.5
Alpine meadow	14.5
Rock crevice	21.5
Rock fissure	26.5
Humus belt	29.5
Rubble	34.5

Molz (**29**) made an extensive and valuable study of the relation of suction tensions and environments. His findings corroborate the views of Ursprung.

Summary

Regarding the nature of osmotic pressure, widely divergent views have been expressed. One theory places the cause of the phenomenon in the greater bombardment of the membrane by the solute particles than by the solvent particles. The other prominent theory considers the solvent as playing the chief rôle, and is known as the hydrostatic theory.

The use of the term "osmotic pressure" does not imply that the kinetic theory must necessarily be adhered to.

Osmotic pressure is defined as the difference of pressure on solution and solvent, which produces a condition of equilibrium, such that there is no tendency of the solvent to flow in either direction.

VAN'T HOFF inclined to the kinetic theory but did not commit himself. He showed the similarity between the mathematical expression of the gas law and the mathematical expression of the osmotic law. He found the constant to be about the same for both. He used the rather inexact data of PFEFFER as basis for the proof.

The osmotic value of a given cell sap is the molal concentration of an agent which is in dynamic equilibrium with the cell sap. The osmotic value at incipient plasmolysis is symbolized by O_g, at normal by O_n, and at saturation by O_s. It is convenient to append the formula for the agent employed, *e.g.*, $O_g(C_{12}H_{22}O_{11})$.

For each osmotic value there is an equivalent suction tension of the cell sap. The suction tensions for the different stages are symbolized respectively, S_{i_g}, S_{i_n}, and S_{i_s}.

The isosmotic coefficient of a salt is the number in the series established by DE VRIES, which expresses roughly the osmotic activity of the substance, and the degree of dissociation.

The osmotic coefficient of a salt is the ratio of the osmotic value of a cell sap, in terms of cane sugar, to the osmotic value of the same, in terms of the given salt. It is symbolized by *i*, *e.g.*,

$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}.$$

The osmotic value of a cell may change under the influence of external factors.

The osmotic value of neighboring cells need not be the same, even though they belong to the same tissue. The average value for a given tissue is characteristic for that tissue.

No regular gradient of O_g could be established in the direction of the streaming of the water in the plant.

The suction tension of a cell represents the positive or negative pressure which causes the influx of the water into the cell. The use of the term does not imply that the hydrostatic theory is adhered to. This term should be used instead of URSPRUNG's "suction force." The numerical value is given by URSPRUNG's law which states that the "suction force" of a cell equals the suction force of the contents of the cell less the wall pressure. It is symbolized by

$$S_z = S_i - W$$

The relations of these quantities are conveniently represented by graphs first employed by HÖFLER.

There are various methods used for the determination of suction tensions in cells, tissues, or entire plant sections.

There is a suction-tension gradient in the direction of the streaming water in the plant.

The suction tension is influenced by external factors. The moisture of the soil and the relative humidity of the air are the most important.

The suction tension of the root hairs may be used as an indicator for determining the relative resistance which soils offer to the influx of water into the plants.

The suction tension of plants depends upon the habitat.

According to URSPRUNG there can be a polar differentiation of suction tensions in certain cells.

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PLANT NUTRITION STUDIES IN RELATION TO THE TRIANGULAR SYSTEM OF WATER CULTURES*

HARRY F. CLEMENTS

(WITH SIX FIGURES)

A review of the literature bearing on the subject of plant growth in culture solution reveals the fact that criteria used in determining the optimum combination of salts have consisted of measurements of the external portions of plants. Length of tops and roots, green and dry weights of tops and roots, amount of water of transpiration, etc., have served as the means of describing the plants grown in the cultures by the various investigators.

In the course of this article, the term "best" plant will be encountered repeatedly. In order to define the term, it is necessary to consider the purpose for which the plant is grown. Obviously, a plant which is considered best for forage purposes will not be the best so far as seed production is concerned, and *vice versa,* but the very fact that some plants are grown for forage and others for seed, implies that they were capable of being developed in the one direction or the other by man. Thus, when a phenomenon, such as the physiological balance of salts for any particular plant, is to be studied it seems only logical that the plant should be considered from the viewpoint of the plant itself and not from that of the animal. Thus, the general term "best" or "normal" plant refers to one which, more specifically, produces sufficient vegetative growth to insure reproduction of the highest order.

With such a plant in mind, it will be well to consider the indices of normal plants which have been mentioned previously. Measurements of roots must yield conflicting results since GARNER and ALLARD (3) have shown that roots often respond with increased vigor to stimuli which suppress top growth. Obviously, roots can not provide for optimum reproduction, and since the tops are concerned with the survival of the species, attempts must be made to obtain measurements from them. Likewise, if roots become a limiting factor, that condition will be manifested in the expression of the tops.

The amount of transpiration water has been used as an indicator. Here, too, it seems certain that many obstacles will be encountered which will tend to confuse rather than to explain results. At best, the amount of water

* Published with the consent of the Experiment Station Director.

which a plant will lose will depend on a great multiplicity of factors, such as the concentration of the culture solution, succulence of the plants, etc. It seems improbable, therefore, that such a criterion could serve the office of determining what constitutes a good plant.

When such measurements as length and weight of tops are taken, again the question arises: "Is the tallest or the heaviest plant necessarily the best one?" Apparently it is not. Thus, any plant which shows a tendency toward succulence will in all probability be taller than one which shows a tendency to be higher in dry material. Green and dry weights would be affected in a similar manner.

It becomes apparent that any attempt to determine a well balanced plant —one that develops its usual functions normally—must be based not only upon quantitative data but must also take into consideration something other than the external expression of the plant.

The possibilities of using measurements of the external features of plants as standard criteria, therefore, seem to be quite thoroughly exhausted. It is probable that criteria based on the combined measurements of the internal and external features will yield more fruitful results. KRAUS and KRAYBILL (**5**) have pointed out that the external appearance of a plant is simply an expression of an internal condition. GARNER and ALLARD (**3**), on the other hand, have demonstrated that the behavior of plants depends to a large degree on the relative length of light exposure. NIGHTINGALE (**8**), however, emphasized the fact that although light exposures of various durations modify the behavior of the plant, they first cause a change in the internal composition of the plant which then is followed by a response in external appearance. In view of these tremendously important generalizations, it seems that by combining measurements of both the external and internal conditions, a criterion of what constitutes a normal plant may be established. Any attempt to demonstrate that one's best plant must be his tallest, or his heaviest, from either a green weight or dry weight basis would probably end in failure as the result of unreliable criteria. True, there are cases in which a plant may be an excellent one and be the tallest of a series, but when groups of workers differently located and with different points of view use the same measurements, only confusion from the reader's point of view can be the result.

No attempt will be made here to present an extended review of the literature on this subject. TOTTINGHAM (**13**) in 1914 gave a thorough review of the literature in his paper, and since that time several hundred papers have appeared dealing with water cultures. Many phases of plant development and behavior have been approached and many valuable and interesting results obtained. Such topics as the effect of the H-ion concentration on growth, the absorption and effect of certain ions or salt combinations,

rate of absorption, and the application of biometry seem to have attracted the greatest share of interest. Many times the data of one man have seemed to be diametrically opposite to those of another who used exactly the same plant and approximately the same conditions of temperature and humidity. Some have supposed that external conditions could not be duplicated and have gone into the work of perfecting control chambers. It seems improbable that any system having such a great range of salt proportions could be disrupted by a few degrees difference in temperature or a few per cent. difference in humidity. If this were the fact, the triangular system would be absolutely useless in our research problems and all that could be obtained with this technique would be to show the uniqueness of the method itself without any illuminating facts concerning the growth and behavior of plants. It does, however, seem that the relative length of day has not been given proper consideration in this type of experimentation.

Thus, an attempt will be made in this paper to throw light on the organic nutrition of the plants grown in the triangle and to study the internal conditions in relation not only to the various combinations of salts, but also to the various exposures of light.

Methods

Seed treatment

The Scotch Beauty field pea was used in this experiment for no other reason than that legumes, with few exceptions, have not been used in this type of experimentation. In order to obtain uniform seeds, the peas were selected according to their weight. Several hundred seeds were weighed separately, the distribution of these weights was obtained and the two central classes were established from the data. Approximately five thousand seeds were then selected ranging in weight from 0.2193 to 0.2445 grams. Before planting, these seeds were treated in a formalin solution (1:250) for thirty minutes, washed thoroughly in distilled water, and allowed to soak in distilled water for ten hours. They were germinated between folds of moist blotting paper in pans which were kept at a temperature ranging from 29° to 31° C. When the roots were approximately 2.5 cm. long the seedlings were transplanted to the culture jars. These jars were of the glazed crock type and had a capacity of twelve liters. Thus it was possible to grow forty seedlings in a single culture. The importance of large numbers of plants in a culture is obvious, and, according to HIBBARD and GERSHBERG (**4**), necessary. Five seedlings were planted into each of eight corks which were fitted into the cover of the crock.

The solutions used were those having an osmotic pressure of one atmosphere used by SHIVE (**11**). The combinations of salts used were as follows:

$Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4. Iron was added in the form of iron tartrate. It was found that when other iron salts were used they invariably caused a partial precipitation of the other culture salts. Twenty-five cc. of a 0.2 per cent. solution of the iron salt were added to each culture at the time that the solutions were changed. Likewise, an equal quantity of 0.2 per cent. solution of $MnCl_2$ were added to the cultures to overcome a tendency on the part of the plants to be chlorotic.

The twenty-one culture jars were placed in a greenhouse on a rotating table which was kept in continuous operation during the entire experimental period. The solutions were changed once each week, and the experiment was continued for about seven weeks. At the end of each series the following data were obtained: green and dry weights of tops and roots, respectively, length of tops and roots, and various forms of carbohydrates of the tops, ash content of tops and roots, total nitrogen of the tops, and the nitrate content of both roots and tops. As the experiment progressed some of these indices were omitted after it was evident that they would aid little in the interpretation of results. The plants of all three series were free from disease.

Chemical methods

A. Desiccation.—Drying the plants was effected in an oven heated to 90° C. for the first half hour to insure killing all enzymes, after which the temperature was reduced to 65° C., following the recommendations of Tottingham and Link (**6**).

B. Carbohydrates.—Three-gram samples of the dried material were used in the carbohydrate analyses. These samples were subjected to eighteen hours of continuous ether extraction. They were allowed to dry and were then subjected to a two-hour extraction in 90 per cent. ethyl alcohol.

Between the time that series 1 and 2 were run, collateral work with other plants showed that preserving the samples in alcohol instead of drying, considerably reduced the variation one found between two portions of the same sample. Hence, in series 2 and 3, approximately one half of the peas from a single culture was placed in alcohol immediately after length and weight measurements were taken. Such samples were later ground in a plate grinder, more fresh alcohol added, and the whole refluxed for two hours. The rest of the procedure was the same as in those cases where dried material was used.

a. *Simple sugars and sucrose.*—Following the alcohol extraction, the mixture was filtered. The filtrate was evaporated almost to dryness under reduced pressure which was so regulated that the alcohol boiled at never more than 50° C. The residue was taken up in water. Sometimes it was

difficult to get out all of the material and in such cases the water was either warmed or some ether was added. This watery extract was then clarified with Horne's dry lead. Disodium phosphate was used in deleading, following the recommendation of ENGLIS and TSANG (**2**). They find that carbonates, sulphates, and oxalates cause losses of reducing substances while disodium phosphate causes practically no loss. Their findings were also verified in this laboratory. After filtration, the filtrate was diluted to 500 cc.

For the sucrose determinations, aliquots of the above volume were treated according to the chemical method outlined in the Official Methods.

The reducing power of these solutions was obtained by the use of the SHAFFER-HARTMAN (**10**) modification of the MUNSON-WALKER method. Although there seems to be some question in the minds of the Standardization Committee (**1**) of the American Society of Plant Physiologists concerning the use of this method, there appears to be no definite criticism of the method. In this laboratory, the method was compared with the official MUNSON-WALKER method and showed every indication of being the more reliable of the two. Furthermore, in the process of clarification, even after the most severe filtration, there always remained some precipitated lead salt in a fine degree of suspension. After long standing this settles out. If the gravimetric method is used, the solutions must be allowed to stand until this has settled out; otherwise this, too, would be weighed as cuprous oxide. On the other hand, this suspension does not in any way affect the iodometric method. Although the permanganate method is accurate, it entails considerable routine and where many analyses are to be made, time becomes a limiting factor. It was possible in this laboratory for a single person to complete twenty analyses in an hour. It is felt that this method should be given a wider use. So, in spite of the recommendations of the Committee, the SHAFFER-HARTMAN method was used in this laboratory.

b. *Starch.*—The residue from the alcohol extraction was washed off the filter paper with water and the mixture was boiled for five minutes to thoroughly gelatinize the starch; it was then cooled, and 10 cc. of fresh saliva added. The mixture was allowed to digest over night in an incubator heated to 37° C. This digestion is usually complete after a much shorter digestion, but the routine was so arranged that this digestion began at the end of the day and was allowed to continue until the next morning. The mixture was filtered and H_2SO_4 was added to the filtrate in such quantities that the latter represented a 2.5 per cent. solution of the acid. The solution was then heated for 1.5 hours on a boiling water bath. This solution was cooled, neutralized with NaOH, and again just turned to the acid side, after which the reducing power was determined. Although it seems to be the general practice to clarify these solutions, it is not being done in

this laboratory for the one reason that analyses made on aliquots, some of which were clarified and others not clarified, gave no difference in reducing power. It may be necessary to clarify when the gravimetric method is employed, but not when the volumetric method is used.

c. *Hemi-celluloses.*—The residue obtained from the starch filtration was washed off the filter paper with 2.5 per cent. H_2SO_4, so that approximately 400 cc. of the acid were used. The mixture was then placed on a boiling water bath and allowed to remain there for two and a half hours, filtered, neutralized, turned acid, made to volume and analyzed. Here, again, the solution was not clarified.

It may be well here to make a few remarks regarding this almost entirely unknown group of carbohydrates, the hemi-celluloses. In a general way, it is known that pentosans (such as the xylans and arabans), hexosans (such as galactans, mannans, and glucosans), and such mixed polymers as the pectic bodies and mucilages, fall into this group. Beyond this practically nothing is known. In later papers, it will be shown rather definitely that this group is actively concerned with the normal nutrition of plants, and furthermore varies under some conditions of environment as much as starch. The water-holding capacity of all of the members of this group is of sufficient importance to warrant a detailed study of the group. The unfortunate thing about the reports regarding this group is that there are nearly as many methods of extraction as there are reporters. It is true that only relative results can be obtained, but until chemical methods are devised for the quantitative separation of this very important group of reserves, it will be best to adopt a uniform means of extraction, so that one worker's results may be compared with those of another.

C. Nitrogen.—a. *Nitrate nitrogen.*—Nitrate nitrogen determinations were made on aqueous extracts from three-gram samples of dried material. Devarda's method was employed.

b. *Total organic nitrogen.*—Total nitrogen contents were determined according to the Official Kjeldahl method, using three-gram samples.

D. Ash.—Ash determinations were made on two-gram samples. The dried material was placed in alundum crucibles, thoroughly mixed with 10 cc. of glycerol alcohol (1:2), and incinerated until subsequent weighings showed no further losses.

Conditions of the experiment

Three series of peas were run. Series 1 was started on the 29th of January, 1926, and discontinued March 20. Series 2 was used as a check on series 1, and was run from February 4, 1927, until March 24. Series 3 was begun immediately after the second series was completed. It ran from March 26, 1927, until May 24. Light from eleven 200-watt lamps was pro-

vided for series 1 and 2 from four o'clock in the afternoon until midnight of each day. Series 3 was grown under the normal conditions of light. In other words, series 1 and 2 were grown under long day conditions while series 3 was grown under a shorter light exposure; an exposure, however,

SERIES 1

3.085
3.625 2.956
3.428 3.030 3.236
3.908 3.480 3.536 3.495
3.362 3.231 3.072 3.150 3.485
3.070 3.442 3.350 3.225 2.977 2.588
AVE. GREEN WT. PER PLANT-TOPS

.280
.319 .260
.344 .273 .290
.327 .300 .339 .326
.327 .292 .299 .296 .342
.302 .336 .314 .315 .297 .271
AVE. DRY WT. PER PLANT-TOPS

1.825
1.948 1.733
1.999 1.814 1.774
1.933 1.925 1.921 1.868
1.858 1.718 2.002 1.775 1.835
1.575 1.638 1.626 1.698 1.578 1.430
AVE. GREEN WT. PER PLANT-ROOTS

.087
.094 .088
.102 .091 .088
.097 .093 .094 .099
.082 .078 .107 .090 .096
.066 .076 .077 .089 .082 .069
AVE. DRY WT. PER PLANT-ROOTS

45.5
54.8 48.6
58.7 52.3 51.6
58.5 56.0 56.5 57.4
53.4 53.9 52.3 53.5 54.9
58.5 56.9 57.8 55.8 53.3 47.8
AVE. LENGTH (CM.) PER PLANT-TOPS

29.2
28.6 31.9
28.2 27.0 27.3
30.4 27.6 28.6 25.9
29.7 26.6 27.8 27.4 24.2
30.0 30.4 26.8 25.0 26.5 24.0
AVE. LENGTH (CM.) PER PLANT-ROOTS

1.29
1.38 1.45
1.35 1.31 1.57
1.47 1.37 1.41 1.31
1.35 1.27 1.25 1.48 1.51
1.25 1.33 1.27 1.26 1.20 1.08
NITRATES-PERCENT DRY WT.-TOPS

1.11
1.20 1.28
1.28 1.28 1.44
1.23 1.59 1.62 1.48
1.41 1.42 1.41 1.72 1.43
1.08 1.29 1.45 1.42 1.63 1.42
NITRATES-PERCENT DRY WT.-ROOTS

FIG. 1. Distribution of the various indicated measurements of the peas grown under a long light exposure from January 29, 1926, to March 20, 1926.

that approached the normal day for the growth of peas. The temperature of the greenhouse was kept around 20° C. and the humidity remained relatively low.

As the reader looks at the triangles shown in figs. 1–6, the left hand corner represents the high $MgSO_4$ corner; the right hand corner the high

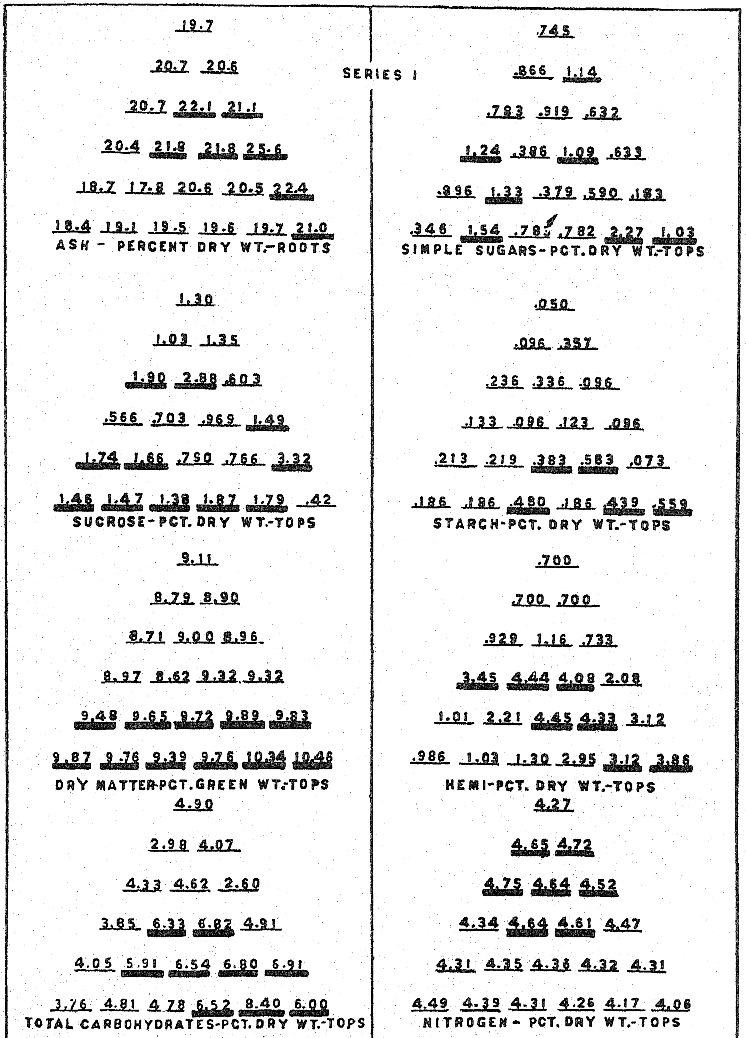

FIG. 2. Distribution of the various indicated measurements of the peas grown under a long light exposure from January 29, 1926, to March 20, 1926.

$Ca(NO_3)_2$ corner; and the top represents the high KH_2PO_4 corner. The heavily underscored figures indicate the cultures which produced plants highest in that particular measurement.

Results and discussion

It will be interesting to compare the results of series 1 and 2. These were really checks on one another and should compare favorably. When

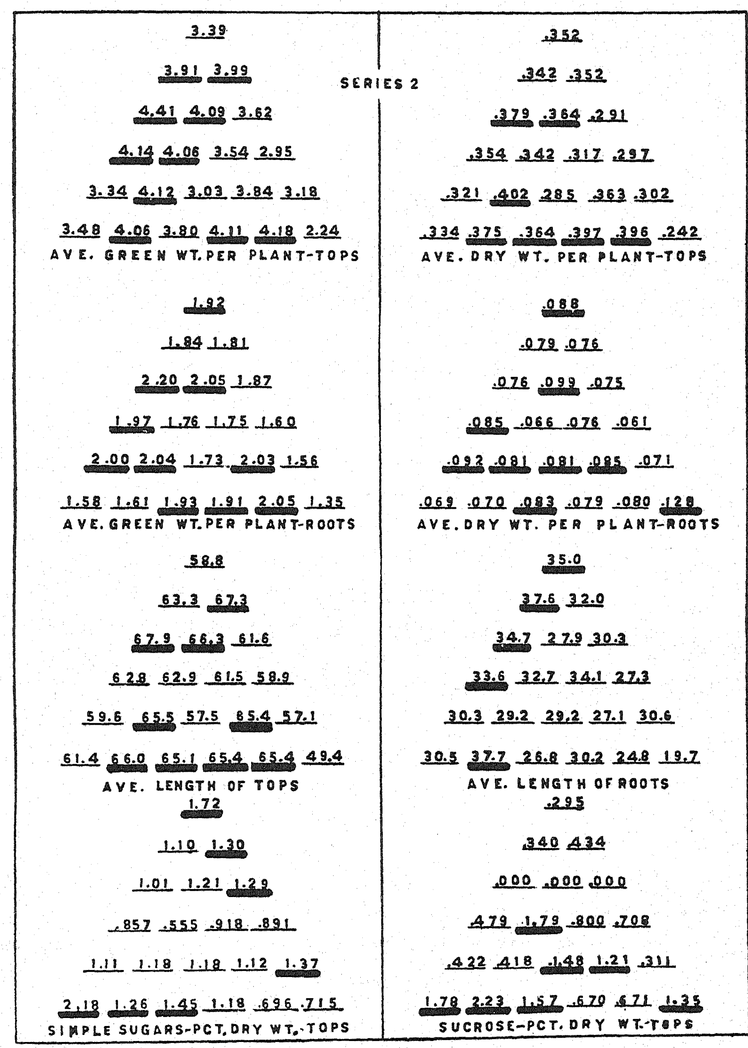

Fig. 3. Distribution of the various indicated measurements of peas grown under a long light exposure from February 4, 1927, to March 24, 1927.

one considers the length of the tops and roots of the two series (figs. 1 and 3), it is doubtful whether or not he would be favorably impressed

since there is a lack of similarity and yet there seems to be a tendency for the highest plants to fall in the same side of the triangle, that is, on the left hand side. When the green weights are examined (figs. 1 and 3), there is a much closer resemblance between the two series, but even they do not check in the way one would like to have them. Dry weights, likewise, tend to be the highest in certain regions and yet they are quite different. This difference may be due to the fact that there were more cloudy days in the period in which series 1 was growing than in that in which the second series was run. It thus becomes obvious that a different means of measuring the plants must be employed. Nitrate determinations (figs. 1 and 3) reveal little in the way of tangible results. In the tops, the highest nitrate contents were found more or less scattered over the triangle. This is to be expected since there are several things which would account for the variability, such as rate of absorption, rate of assimilation, etc. The nitrates of the roots seem to be most abundant in the high $Ca(NO_3)_2$ corner, and this might be expected, but surely this cannot be a measuring stick.

As the next attempt, the ash content of the roots was determined, and although the highest ash content centered on six cultures, it is still a question whether this would indicate good or inferior plants (fig. 2).

Sugar determinations seem to be more promising (figs. 2 and 3). Simple sugars do not yield any definite information except that little can be determined from them. This will be emphasized even more in a report to follow later. Sucrose likewise is erratic, although it shows a strong tendency to be highest in the lower part of the triangle. Starch (figs. 2 and 4) behaves in a similar manner in both series. Hemi-celluloses, too, seem to be most abundant in the lower part of the triangle. Total carbohydrates, that is, the sum of all the carbohydrates reported plus dextrins, are decidedly localized in the lower part of the triangle, and what is more, *they are the most abundant in plants grown in solutions which have the highest proportion of nitrates.* This, indeed, is a startling finding.

When total nitrogen determinations of the tops are examined (figs. 2 and 4), an extremely interesting thing is found. The highest amounts of organic nitrogen, on a per cent. basis, show an extremely well localized area, not where there is the highest nitrate content in the solution, but where there is the highest content of the potassium salt. This finding bears out the study of STOKLASA (**12**). A study of the relation of potassium to nitrogen assimilation under various exposures of light would, indeed, be fascinating. This is especially promising in view of the so-called radioactivity of potassium.

To show further that this question hinges around the nitrogen reserve, it will be well to call attention to another fact. With the gradual variation in the salt combinations used in any one row in the triangle, there must be

one culture which shows better salt absorption than any other culture in the same row. Thus, no matter how good or how poor a series of plants may be there must necessarily be one plant in this series which is either the best or the poorest. So in any row of the triangle one culture must either be the lowest in total nitrogen or the highest. With the gradual variation in the combination of nutrient salts there ought to be a gradual variation

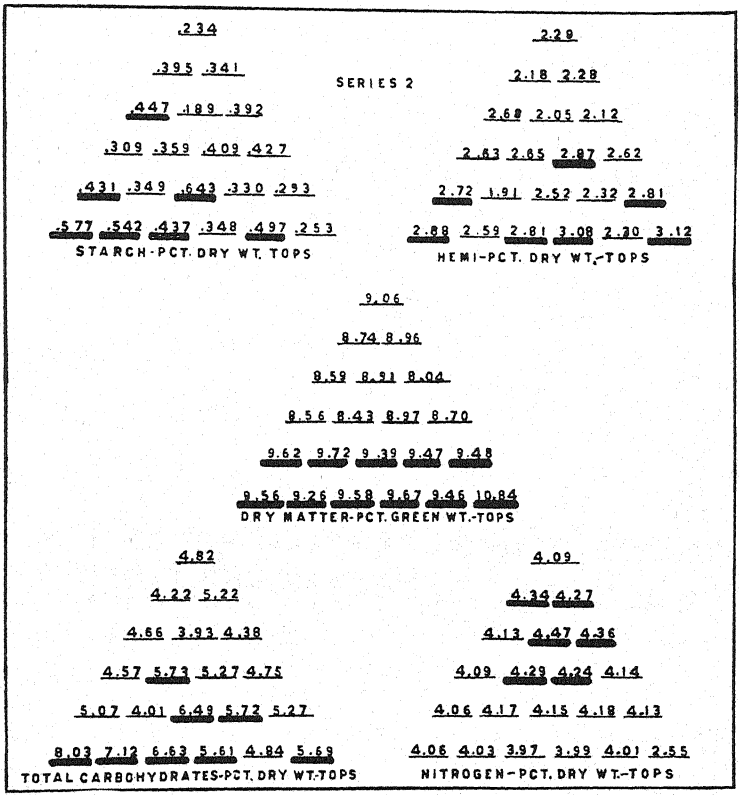

Fig. 4. Distribution of the various indicated measurements of peas grown under a long light exposure from February 4, 1927, to March 24, 1927.

in the total nitrogen contents in either one or two directions. This is especially outstanding in the total nitrogen results of all three series. Thus, the total nitrogen per cent. of series 1, the lowest line, reads 4.49, 4.39, 4.31, 4.26, 4.17, and 4.06. The row leading from the lower left hand corner to the upper corner reads 4.49, 4.31, 4.34, 4.75, 4.45, and 4.27. In the first row mentioned, the highest nitrogen assimilation occurred in the first culture and a gradual but definite decrease is found throughout the row until the last culture. In the second row given, the cultures with one exception in-

creased up to 4.75, and then decreased. It is extremely interesting to compare these trends in series 1 with those in series 2. Although all of the cultures ran lower in total nitrogen in series 2 than in series 1, these trends within the series show marked similarities. Thus in series 1, the high culture in the row on the left side of the triangle was the fourth culture up while in series 2 it was the fifth. In the row on the right, the fifth culture up was the highest while in series 2, the fourth culture was the highest. In the bottom row, the first culture on the left was highest, the one on the right was lowest, exactly as in series 2. It seems that these data show very definitely that the absorption and assimilation of nitrogen are markedly affected by the physiological balance of the culture solution. The trends in series 3 reveal the fact that a shorter light exposure very markedly affects the position of the highest culture in any one row. This seems to yield results favorable to the question asked by NIGHTINGALE and KRAUS (9): "Does light affect the fertilizer requirements of plants?" It seems very apparent that it does.

In a measure, now, we are able to generalize regarding the best culture solution, and regarding what we might term as the best plant or series of plants. KRAUS and KRAYBILL working with the tomato found a very definite relation existing between abundant organic nitrogen and succulence of plants, and between a high carbohydrate content and high dry matter. As either of these two groups of reserves became excessive, the plants no longer were regarded as normal because their reproductive process was hindered. When the plants were extremely high in nitrogen, many of their flowers failed to develop, while the general stature of the plant continued to enlarge. On the other hand, when the plants were extremely high in carbohydrates, some flowers developed and set fruit, but the vegetative growth of the plants was not sufficient to make efficient reproduction possible. Reproduction of the highest order was found only in those plants in which there was neither an excess of nitrogen nor an excess of carbohydrates. In other words, if these two reserves are moderately abundant in plants, the plant will express that internal condition by having vigorous vegetative development accompanied by fruitfulness. This, obviously, is what will be a normal plant. Although the tomato is a highly selected plant which must depend on artificial care for its proper development, it afforded a picture which has been verified time and again with other plants. The pomologist finds the same principles applicable to his fruit trees and regulates the reserve balance by proper pruning, fertilizing, etc. The florist practices it when he continually re-pots his plants until a short time before he wants them to flower. Then, he allows the plants to become "pot bound." This is nothing more than limiting root development and nitrate absorption and hence, correcting the highly vegetative state of his plants. The agronomist

realizes the application of the nitrogen-carbohydrate principle when he criticizes a field of grain as being too heavy in straw (too vegetative) or being too short (non-vegetative). The production of grain obviously is optimum when the straw is neither short nor long but intermediate. Thus, it seems only fair that this same criterion should be used in experimental plots and culture solutions.

In the triangles of series 1 and 2, the high nitrogen area is found in the top of the triangle, while the high carbohydrate area is found at the bottom. In view of the relation described above, it becomes obvious that the best plant of the series will not be found in the high nitrogen area, nor in the high carbohydrate area, but must be in a region which is intermediate with respect to these two groups of reserves. This region in the triangle, of course, will be between the high nitrogen and high carbohydrate areas. It was indicated above that plants high in nitrogen are high in moisture and those low in nitrogen and high in carbohydrates will be high in dry matter. A glance at the triangles representing dry matter in series 1 and 2 will not only verify this generalization but will show how closely the two series resemble one another.

The fact that the areas in which we find these reserves in greatest abundance are practically the same, for the first two series show rather decisively that here is a measuring stick which can be used to determine the region of the triangle in which best growing conditions prevail.

Thus, it is readily seen that this new criterion is far more satisfactory in determining the best cultures. Neither the total nitrogen nor the total carbohydrates can serve as indicators when taken alone, but when they are used in relation to one another, very definite information is obtained. Each maintains a definite area in series 1 and 2, and in view of the discussion here presented the best balanced plant cannot be found in either groups, but in the region between these areas. After this region is located, then such indices as length of the plants, their weights, etc., can select the best culture. Following is a diagram showing the numbering system:

```
               21
            19    20
         16    17    18
      12    13    14    15
    7     8     9    10    11
 1     2     3     4     5     6
```

In series 1, cultures no. 13, 14, 16, 17, 18, 19, 20 are eliminated from further consideration because of their high nitrogen content. Cultures no. 4, 5, 6, 8, 9, 10, 11, 13, 14 are eliminated because of their high carbohydrate content. Thus, cultures no. 1, 2, 3, 7, 12, 15, and 21 remain. Culture no. 21 is decidedly mediocre in its external appearance, being the shortest plant of the entire series. Of the remaining cultures no. 12 seems to be superior to the others in that it is high in green weight and dry weight of both tops and roots. Its tops are taller than those of any of the remaining cultures.

In series two, which should approximate series one since light conditions were practically the same, the carbohydrates are most abundant in the lower part of the triangle while the high nitrogen plants are found near the top. Thus, cultures no. (fig. 4) 13, 14, 16, 17, 18, 19 and 20 are eliminated because of their high nitrogen content. Cultures no. 1, 2, 3, 4, 6, 9, 10 and 13 are eliminated because of their high carbohydrate content. Cultures no. 5, 7, 8, 11, 12, 15, 21 remain for further consideration. It is to be noted that of these, 7, 12, 15 and 21 were also found in the remaining group in series 1 after the poorest cultures were removed. Thus, it is possible to select a culture which showed good growth in both series. That there is some difference in the growth of the plants can well be explained by temperature and humidity conditions. Culture no. 21 shows strong tendencies to favor high carbohydrate formation in both series. Culture no. 15 is decidedly inferior in its external features in series 2. Culture no. 12 is superior to no. 7, in green and dry weight of tops and in length of tops and roots, and since no. 12 showed a decided advantage over the other cultures in series 1, it seems only just to select it as the best culture in which to grow peas under a long day exposure. This balance is made up of three parts of KH_2PO_4, one part of $Ca(NO_3)_2$ and four parts of $MgSO_4$.

Now it will be of interest to see what effect a shorter day has on the relative positions taken by the highest carbohydrate and highest nitrogen cultures. Series 3 was run for that purpose. Beginning with the superficial measurements such as length of tops, roots, their green and dry weights (fig. 5), it is seen at once that our biggest plants are in an entirely different part of the triangle than they were in the previous series. Furthermore, when the region in which nitrogen is the highest is examined (fig. 6), it is seen at once that it has in a sense migrated to a lower position on the triangle nearer to the high nitrate corner. How has the high carbohydrate area responded? That has seemingly been switched around so that now instead of being in the lower right hand corner as in previous series, it is found in the left hand side of the triangle. Under these conditions, we must look for the best plant in a place different from that of the other series.

In applying the elimination process to this series (fig. 6), cultures no. 4, 9, 10, 13, 14 and 16 represent high nitrogen cultures. Cultures no. 1, 2, 3, 7, 12, 16, 17, 18, 19, 20 and 21 represent high carbohydrate cultures. Thus, the best plants must be found among cultures no. 5, 6, 8, 11 or 15. Culture no. 6 does not compare favorably with the other cultures so far as the external measurements are concerned (fig. 5). Culture no. 8 is rather

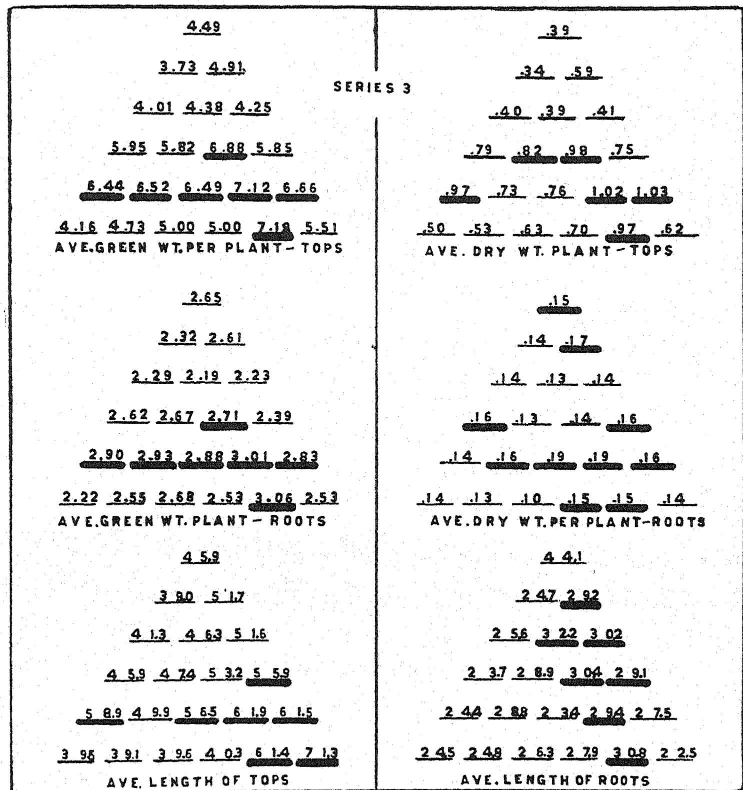

FIG. 5. Distribution of the various indicated measurements of peas grown under a shorter light exposure from March 26, 1927, to May 24, 1927.

high in moisture while culture no. 11 is higher in dry matter than any other culture. Culture no. 15 shows a tendency for greater root development in proportion to tops and thus culture no. 5 is left. It is moderately high in dry matter and nitrogen. Its low content of carbohydrates is well explained by the fact that its top and root development is excellent. Thus, culture no. 5 apparently contains the optimum balance of salts in which to grow peas under an intermediate light condition. This balance is represented by one part of KH_2PO_4, five parts of $Ca(NO_3)_2$, and two parts of $MgSO_4$.

This, perhaps, helps in a measure to explain some of the inconsistencies which are encountered in the literature regarding the plants grown in the triangular system. We are contending not only with a variety of salt combinations but also with the way plants respond to these combinations in building their reserves. These reserves then seemingly dictate the external expression of the plant. Further than that, we are contending with the

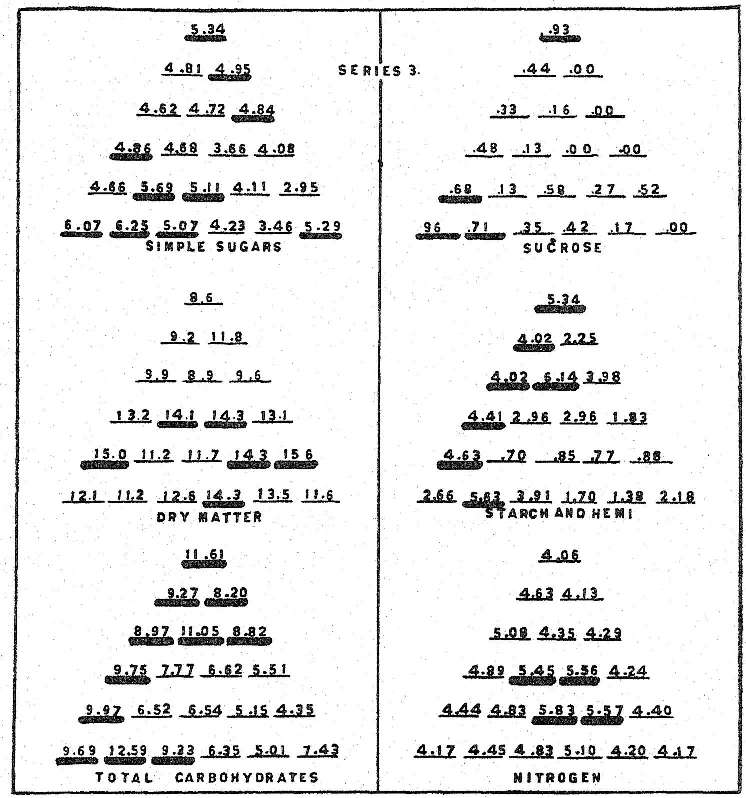

FIG. 6. Distribution of the various indicated measurements of peas grown under a shorter light exposure from March 26, 1927, to May 24, 1927.

effect that light has on modifying the response of plants to the combination of salts.

Following such studies it becomes apparent that the triangular system has the extremely important rôle in plant physiology of showing the effect that light has on modifying the fertility requirements of plants in the various sections of the country and at various seasons of the year. Furthermore, the aid of the triangle will be of inestimable value in studying the effect of the various elements on such functions as nitrogen assimilation and carbohydrate accumulation and utilization.

The writer wishes to express his gratitude to Dr. E. A. BESSEY for his criticisms of the manuscript, and to Dr. R. P. HIBBARD for the use of his laboratory and equipment.

Summary

1. Three series of peas were grown in the triangular system of water cultures. Series 1 and 2 were grown at the same time of the year, the former in 1926, the latter in 1927. Series 3 was run in 1927 but at a date later than series 2.
2. From data presented, it is obvious that such criteria as length of plants, green and dry weights, etc., cannot give a correct idea of the best balance of salts for the growth of plants.
3. Indices such as highest ash content and highest nitrate content are presented, but are shown to be erratic in their positions and hence unreliable.
4. This index unreliability is likewise true of the soluble forms of carbohydrates.
5. Data are presented which show that KH_2PO_4 has a very marked influence on nitrogen assimilation.
6. The areas in which nitrogen and carbohydrates are highest, respectively, assume very definite positions in the triangle.
7. The positions of high nitrogen and high carbohydrate areas are taken at opposite sides of the triangle and leave an area in which the best plants of the triangle may be expected to be found.
8. It is shown that a shorter exposure to light results in different positions being occupied by the best cultures. This must be expected in view of the behavior of the two large groups of reserves found in the plants which were grown under shorter light exposures.
9. A possible rôle of the triangular system of water cultures in future research regarding fertilizer requirements is mentioned.

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ANATOMICAL MATERIAL FOR THE STUDY OF GROWTH DIFFERENTIATION IN HIGHER PLANTS*

RAY BOUILLENNE

(WITH THIRTEEN FIGURES)

As Fauré-Fremiet[1] says very explicitly in his introduction to "The kinetics of development," the morphological study of ontogenesis of the organs and the order of their developmental succession in the individual during the formative period represents a merely external point of view; it offers merely a point of contact. The author does add, however, that morphological indications are as yet of too great importance to be completely neglected.

Our physiological studies on one hand, and certain anatomical investigations on the other, made during a botanical expedition[2] in the Brazilian Amazon, have by their coincidence made it possible for us to furnish anatomical material for a study of growth differentiation.

We wish to draw attention to a singularly interesting case which illustrates that morphological considerations are able to bring us to the same point as purely physiological results are doing.

Physiological studies of growth phenomena have permitted us to perceive the relations between growth and age, between growth and the chemical composition of the plant organs and the correlations existing in the development of the different organs. It has proved possible to trace curves expressing mathematical laws. These have the same general trend in plants as in animals. The majority of authors have recorded their observations in successive measurements of weight, of volume and of length during the course of development; for example, the works of BRODY[3] on the growth of the domestic fowl, BRODY and RAGSDALE[4] on the cow, and those of PRIESTLEY, PEARSALL and EVERSHED[5,6] on *Tradescantia* and other plant roots.

* Translated from the original French manuscript by DR. MARY JUHN, research assistant in zoology, the University of Chicago.

1 FAURÉ-FREMIET, E. La cinétique du développement. Les Presses Universitaires de France. Paris. 1925.

2 Belgian botanical expedition in Brazil, 1922–1923, organized by the Belgian Government and the "Fondation Universitaire."

3 BRODY, S. The rate of growth of the domestic fowl. Jour. Gen. Physiol. 3: 765–770. 1921.

4 ————, and RAGSDALE, A. C. The rate of growth of the dairy cow. Jour. Gen. Physiol. 3: 623–633. 1921.

The observations at our disposal on the morphological variations correlated to these modifications of weight, of length and of volume, though of the greatest importance, are very few in number. In zoology, for example, descriptions of embryonic developmental phases and of metamorphoses have been furnished since a long time. To-day the attempt is being made to find the physico-chemical reasons for the processes of differentiation and a distinction is made between the part due to heredity and the part due to actual causes (causative embryology). Valuable arguments in histology and in cytology have been furnished by CONKLIN's paper on the nucleo-cytoplasmic relation during the course of development. These findings have been amplified by ROBERTSON in his book, "Chemical basis of growth and senescence."

Botanical literature, however, appears to contain little information concerning the different morphological stages through which a plant passes during its developmental period. I do not at all wish to discuss the adaptations or accommodations to actual conditions such as have, for example, been described by MASSART for *Polygonum amphibium*, but rather to consider the variations in structure during the normal developmental period. The reason for this is that plant embryology can only be considered from a very special point of view, and this point of view differs considerably from the one used in animal embryology.

Thus metazoan embryology ends with the recognition of a young individual whose general organization is, so to speak, established and which will subsequently undergo only modifications of size, of maturation, and senescence. In the plant on the contrary, the stage of embryonic differentiation does not terminate with the formation of the embryo and the ripening of the seed. The meristematic tissues of the stems and the roots of plants continue to function as generative tissues during the entire life of the plant; there is a kind of prolonged embryonic condition which is confined to certain regions, at the extremities of stems and roots, and to the cambium. These meristems appear to be exposed at every moment of the plant's life to the internal conditions of heredity, ontogenetic evolution and to the external conditions of the environment.

These generative tissues, but undifferentiated ones, give rise to primary tissues within which structures become established; and so appear new regions of stems and roots.

We know that every part of a stem, for instance, has its own structure, varying within the limits of the species. Are we able to conclude from these variations that meristem has different potential qualities?

[5] PRIESTLEY, J. H., and PEARSALL, W. H. An interpretation of some growth curves. Ann. Bot. **36**: 239–249. 1922. (See also Ann. Bot. **36**: 485–488. 1922, and **37**: 261–275. 1923.)

[6] ————, and EVERSHED, A. F. C. H. A quantitative study of the growth of roots. Ann. Bot. **36**: 225–237. 1922.

The question proposed in this paper is as follows: *Are there any morphological proofs for different potentialities of the meristem during the normal growth phenomena? Is it possible to find structures whose succession, along a stem or a root, is parallel to the physiological variations of growth?*

Our intention is not to make a study of the relations between the physicochemical phenomena of growth in plants and their forms and structures but rather to add new material to the knowledge of these correlations; in other words to give these latter an interesting morphological illustration.

Description of material

Up to the present time, the leaves, stems, branches and inflorescenses have been the only parts of higher plants to furnish information on the subject of correlations between growth and structure. As to roots, they generally show no important variations; they have a very uniform anatomical structure in all vascular plants and this structure is identical or constant along the long axis. In the Monocotyledons as well as in the Dicotyledons the root consists of a central cylinder, forming in cross-section a perfect circle, limited at the external periphery of the central cylinder by an endodermis and a pericycle. On this curve is oriented a large number of vascular bundles, and the whole is surrounded by the cortical parenchyma. In the young stages one can scarcely distinguish the root of Monocotyledons from those of Dicotyledons except perhaps by the greater number of vascular bundles in the former. In the adult Dicotyledon a cambium tissue modifies the structure. The Monocotyledons which have no cambium retain their diameter and primitive organization.

We have had occasion to study from a purely anatomical viewpoint a very interesting case of variation, the "stilt" roots of the Amazon Palm, *Iriartea exorrhiza* Mart. We believe it will be interesting to present the facts, freed from the purely anatomical considerations.[7]

Iriartea exorrhiza Mart., a ceroxylian palm, lives in the forests of Varzea[8] along the banks of the Amazon in very muddy soil which is inundated during the greater part of the year. The palm, fig. 1, has large, stilt-like aërial prop roots which take their origin from the stem at different nodes and which enter the soil at an oblique angle. This arrangement constitutes an unique case among the palms and is very rare among the Monocotyledons.

We will describe the structure of these roots in young palms of different ages and in the adult; this will show the different peculiarities of root structures from germination.

We find that the roots are inserted higher and higher up on the lower region of the stem, and that the most recently formed aërial roots in the

[7] BOUILLENNE, RAY. Les racines-échasses de *Iriartea exorrhiza*. Mem. Acad. Roy. Sci. Belgique. 1925.

[8] Varzea = alluvial prairies or forests.

adult palm are given off at more than two meters from the soil. The roots are not all of the same diameter. Those inserted nearer the soil have a smaller diameter than do the upper roots.

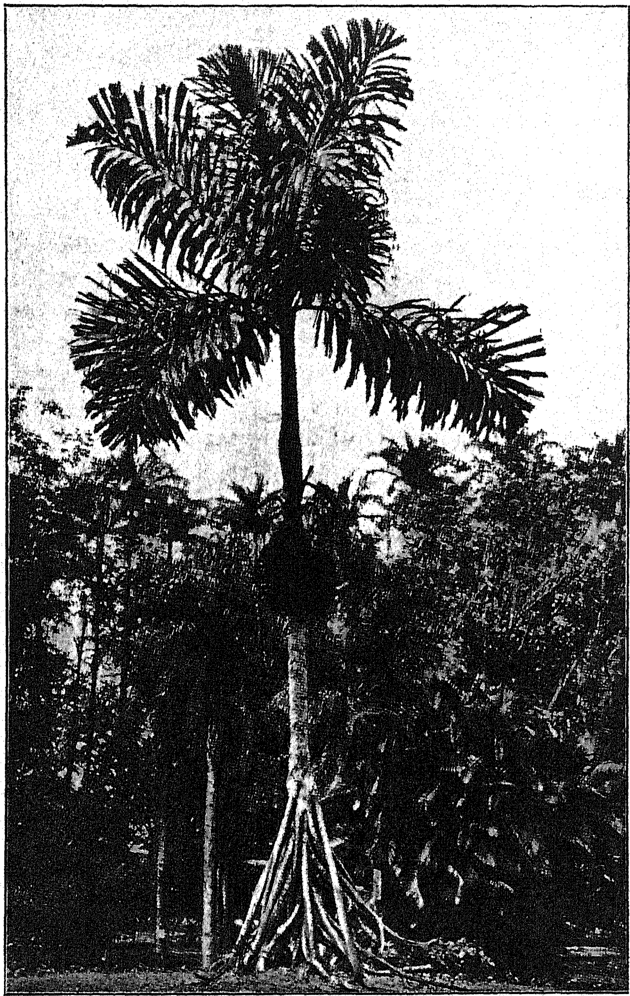
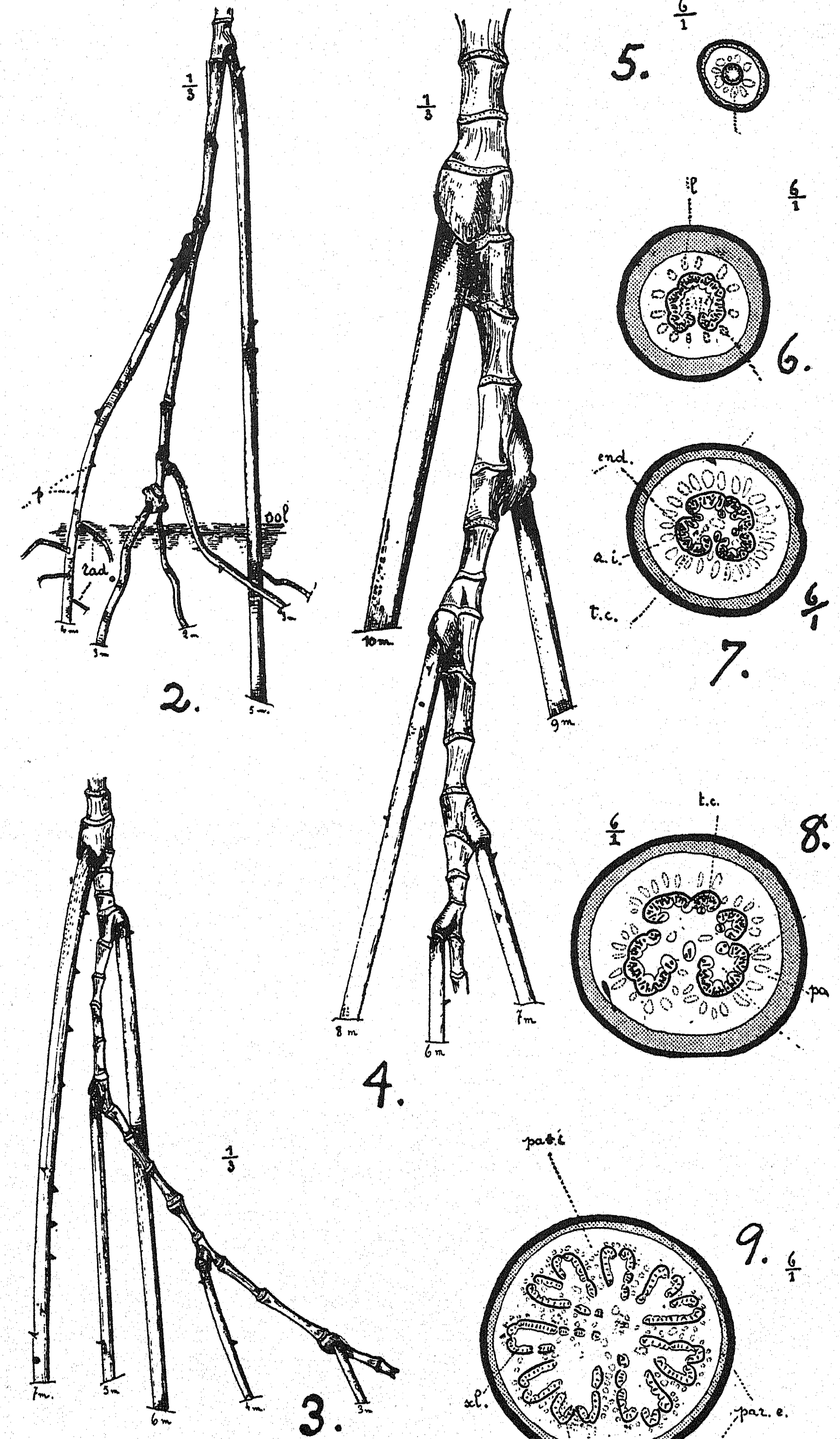


FIG. 1. *Iriartea exorrhiza* Mart. (Palm), from the Botanical Garden, Rio de Janeiro, showing the prop or stilt roots which arise at different levels from the base of the stem. The inflorescence is visible beneath the leaf sheaths at the apex of the stem.

I. Young plants

The entire series of observations made can be demonstrated from two of the numerous young plants that we collected in the Varzea forests. We



2.
1/3
p
sol
rad.
4m
3m
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5m
3.
1/3
7m
5m
6m
4m
3m
4.
1/3
10m
9m
8m
7m
6m
5.
6/1
6.
6/1
l
7.
6/1
end.
a.i.
t.c.
8.
6/1
t.c.
pa
9.
6/1
par.i
xl.
par.e.

FIG. 2. Base of a young plant, 40 cm. tall, showing five adventitious roots, inserted at different levels on the stem, and having respectively from lowest to highest 2, 3, 3, 4, and 5 mm. diameter. Magnification, $\frac{1}{3}$.

FIG. 3. Base of a young plant of *Iriartea* showing the increasing diameter of the "stilt" roots with higher insertion on the stems.

FIG. 4. Portion of a young plant, one meter tall, showing five of its adventitious roots with 6, 7, 8, 9, and 10 mm. diameter respectively. Drawn $\frac{1}{3}$ natural size.

FIG. 5. Cross-section of a primary root of *Iriartea,* 2 mm. in diameter. See description in text.

FIG. 6. Cross-section of a root 4 mm. in diameter. Thirty-one xylem bundles. See text. l = aerial lacunae.

FIG. 7. Cross-section of a 6 mm. root, showing 62 xylem bundles. See text. end = endodermis.

FIG. 8. Cross-section of a slightly larger root, showing the same features as fig. 7, but intercellular spaces more pronounced. t. c. = conjunctive tissue.

FIG. 9. Cross-section of a 10 mm. root, having 229 xylem bundles. See text for description.
par. e. = external cortical parenchyma.
par. i. = internal cortical parenchyma.
scl. = sclerenchyma.

will therefore limit our description to these two, the youngest and the oldest, of the entire lot.

The youngest plantlet (fig. 2) measures 0.4 m. in length and has three leaves, the narrow stem having a diameter of 2.5 mm. at the base and 10 mm. at the apical end. The diameter increases regularly from the base to the tip. The basal part of the stem itself has two little roots, one of which is the principal root. Adventitious, aërial roots have been given off singly at some of the nodes, quite close to the leaves.

These aërial roots appear therefore at different levels; they cause an extensive rupture of the epidermis a little beneath the nodes. These five aërial roots have diameters measuring 2, 3, 3, 4, and 5 mm., respectively. These measurements have a direct relation to the diameter of the stem at the point of their insertion, in other words, the nearer the point of insertion of the aërial root to the apical end of the palm, the larger is its diameter. See table I. This observation is equally true of the plantlet (fig. 3).

The oldest of the lot of young plants (fig. 4) measures one meter in length. The diameter of the stem in the region near the leaves is 20 mm.; the five aërial roots formed in this region measure from below up 6, 7, 8, 9, and 10 mm. in diameter. As one can see, there is a regular gradation in

the thickness of these roots. This observation is confirmed by data from other plants, which will not be presented here, but which are incorporated in the graphic data of fig. 10 and table I.

TABLE I

DIAMETER RELATIONS OF ROOTS AND STEMS

DIAMETER OE ROOTS	DIAMETER OF STEMS	NODES COUNTED FROM PRINCIPAL ROOT UP
mm.	mm.	
2.0	2.5	1st
3.0	3.0	2–3rd
4.0	4.0	7th
4.5	....	....
5.0	5.0	9th
6.0	6.0	16th
7.0	7.0	....
8.0	9.0	....
9.0	9.0	....
10.0	10.0	....

It is by no means possible to demonstrate sharply this correlation of stem and root diameters on larger parts of stems and on adult plants. On the one hand, there is a greater number of roots appearing at nearly the same point and modifying the true diameter of the stem; on the other hand, roots are lacking on the upper parts of stems.

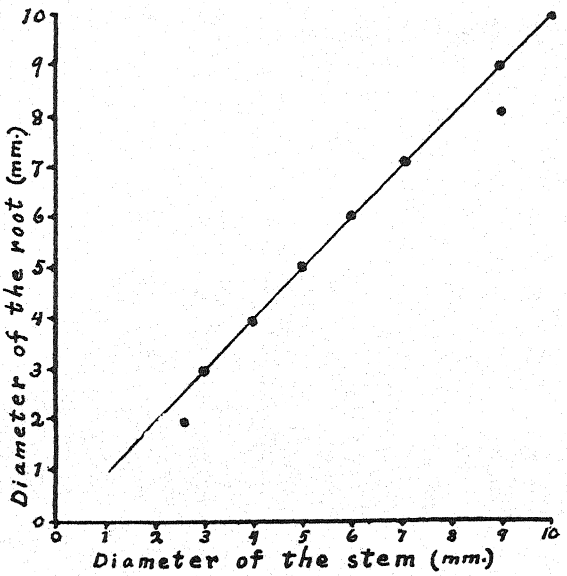


FIG. 10. Correlation of stem and root diameters.

Anatomy of the different roots of the young plants

Cross-sections were made through the roots of these two plants at a distance of 5 cm. from the stem. This level corresponds to the aërial parts. The narrowest root, the primary root, is 2 mm. in diameter and has a true central cylinder, forming a *perfect circle and having 12 xylem bundles alternating with 12 phloem bundles.* The structure of this root can in no wise be distinguished from that of a normal Monocotyledon (fig. 5).

The root measuring 4 mm. in diameter and inserted at the seventh node, approximately at a distance of 10 cm. from the soil, reveals an extremely interesting condition of the central cylinder. 1. The central cylinder is larger than that of the root just described; it has 31 xylem bundles and 33 phloem bundles. 2. The peripheral outline of the central cylinder is wavy. 3. The central cylinder is ruptured (fig. 6).

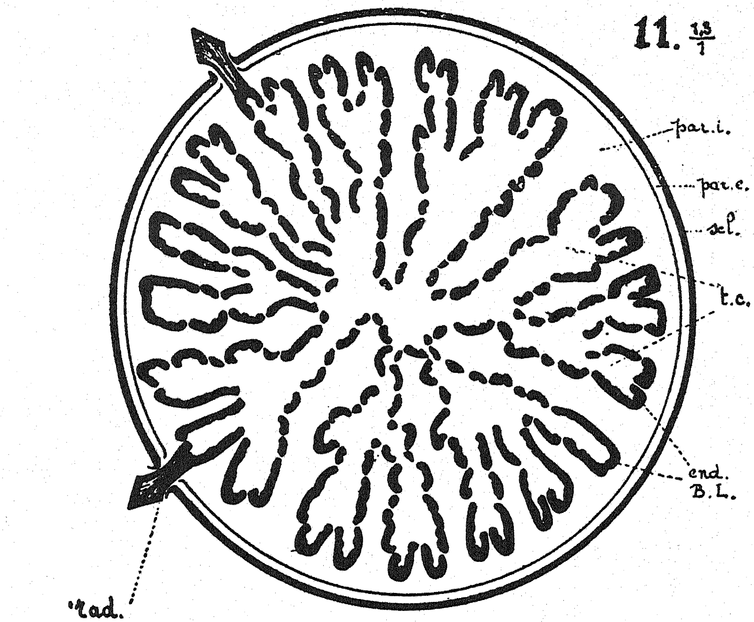
The root measuring 6 mm. in diameter (fig. 7) and inserted in the plantlet at the sixteenth node, at a distance of about 20 cm. from the soil is characterized by the same three differences described for the 4 mm. root, but in a more marked degree. 1. There are 62 xylem bundles forming a yet larger central cylinder and 63 phloem bundles. 2. The wavy outline is even more distinct, and the undulations are of different sizes. The most important ones, five in number, take the form of lobes, within whose curves the xylem bundles are oriented. These five lobes are wavy in outline as well. 3. The central cylinder is ruptured in several places so that it appears as if divided into segments. There are five segments corresponding to the five principal lobes, the edges of these segments are more or less separated from each other by parenchyma (fig. 8).

The root measuring 10 mm. in diameter and inserted in a thick node at 50 cm. from the soil shows the following arrangement: 1. There are 229 xylem bundles. 2. The central cylinder is deeply lobed. Each of these lobes is itself bi- or trifurcate, and these secondary lobes are also separated from each other by strands of parenchyma. The lobes themselves are ruptured in several places, giving the appearance of segments of different sizes.

The large roots of the adult plant

The large aërial roots of the adult *Iriartea* (figs. 11 and 12) are inserted very high up on the stem and their diameter varies from 50–60 mm. This diameter remains approximately constant in the entire aërial region. A cross-section through such a root shows a peculiar design, occupying the entire section, a kind of star with blunt rays. These lobes, of different sizes, are separated from each other by deep sinuses, and are themselves formed by smaller secondary and tertiary lobes. The contour of the lobes is not continuous but broken up into numerous segments of various sizes.

11. $\frac{13}{1}$



12. $\frac{3}{1}$

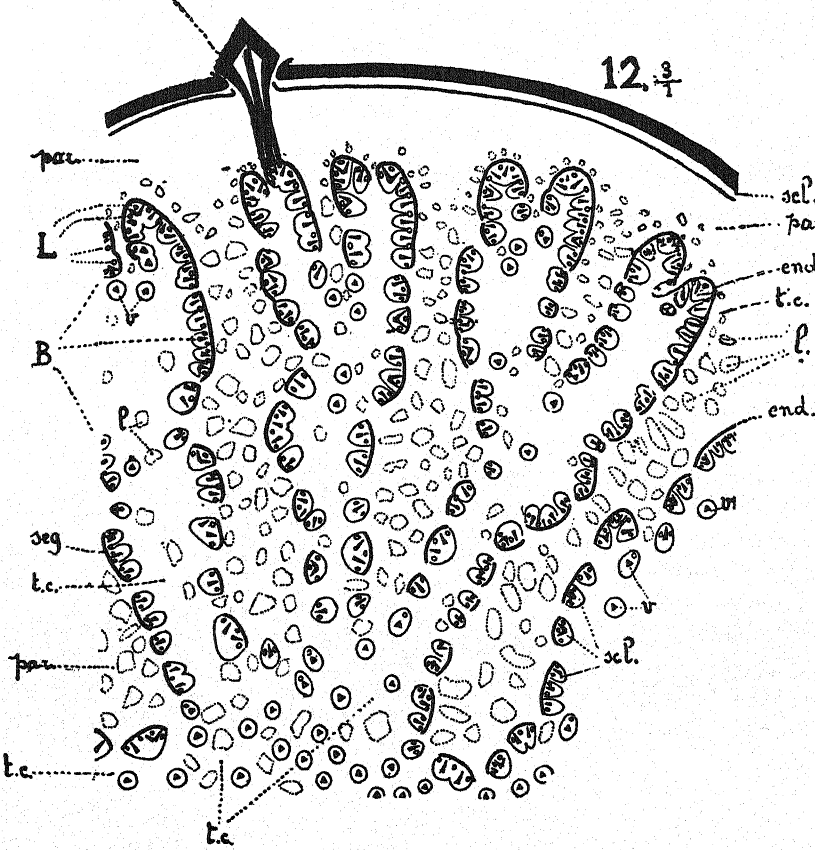


FIG. 11. Cross-section, ×1.3, of root of adult *Iriartea exorrhiza.* Diameter, 5 cm. The central cylinder is visible, and shows the star-shaped outline formed by its lobes and segments. Two thorns, sclerified radicles, may be seen.

FIG. 12. Single lobe of the central cylinder, ×3. One can distinguish the elements constituting the lobes and segments.

B = xylem, indicated by black arrows.
L = phloem, indicated by small white circles.
end. = endodermis.
I. = intercellular spaces.
par-e. = external cortical parenchyma.
par-i. = internal cortical parenchyma.
rad. = sclerified radicle, forming a spiny projection.
scl. = sclerenchyma.
seg. = a segment.
v. = an isolated vessel surrounded by sclerenchyma; the vessel is indicated by a black triangle.

The star-shaped design is composed of xylem bundles that are arranged perpendicularly to its outline, each composed of 3–5 tracheae and one or two large vessels. The number of these xylem bundles is quite exceptional, being 1300–1500. The phloem bundles usually, though not always, alternate with the xylem and are present in even greater numbers, 1600–1800. To our knowledge such a complicated structure has been described only in the roots of *Iriartea.*[9] It is easily explained from transition stages revealed by the study of the young rootlets, although first studies on the adult plant did not lead to an understanding of its root nature.[10]

These different stages are not to be considered as stages in the evolution of the structure of a root in the course of its development. In the roots of the Monocotyledons, the diameter once attained, the structure once established, remains without change. Each of the roots as it is given off from the stem has a structure such as we have described, and retains it during its entire existence.

Discussion

I. In studying the structure of the roots of *Iriartea exorrhiza* Mart., one finds that the central cylinder shows modifications, lobing and breaking up into segments, the degree of complication being directly related to the width of the diameter. Another very remarkable peculiarity of the structural aspect of the root sections is the number of xylem and phloem bundles. The number of these bundles varies, being greater the larger the root and the more complicated the structure of the central cylinder. See tables I and II. The number of the bundles, the structure of the central cylinder

[9] BOUILLENNE, RAY. Les racines-échasses de *Iriartea exorrhiza* Mart. (Palmiers) et de *Pandanus* div. sp. (Pandanacées). Mém. Acad. Roy. Sciences Beligique. 8: 1925.

[10] MOHL, ————. Historia naturalis Palmarum. Martius, vol. 1. 1823.

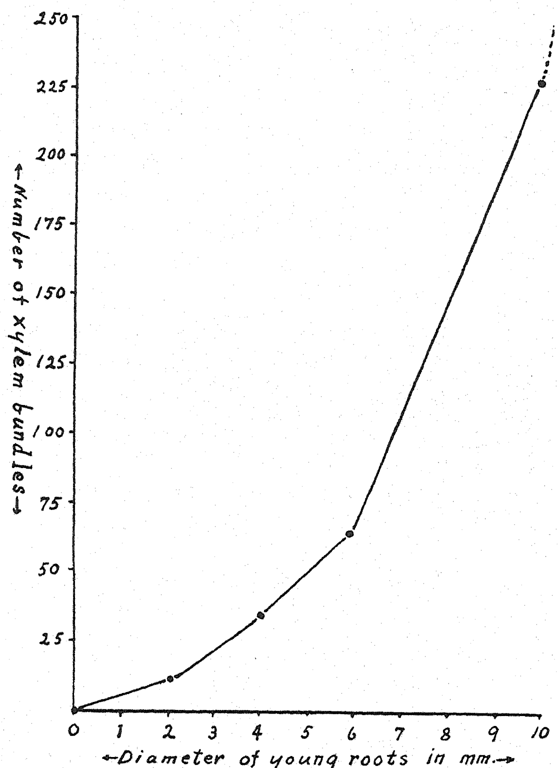


FIG. 13. Graphical representation of the relation of root diameter to the number of xylem bundles.

TABLE II

RELATION OF NUMBER OF VASCULAR BUNDLES TO ROOT DIAMETERS

NUMBER OF VASCULAR BUNDLES	DIAMETER OF THE ROOTS
	mm.
12	2.0
31	4.0
42	4.5
63	6.0
229	10.0
1300	50.0

and the increase in width of the diameter of the root are parallel to each other in their changes from one root to another.

II. When one follows the development of the plant from the beginning of germination, one observes that the first roots formed on the plant are

narrow and simple in structure, and that the roots which appear later during the growth period of the stem are more and more complicated; finally, that the most recently formed roots, which appear on the adult palm, are the largest and have the most highly differentiated structure. We find therefore, from node to node, from the basal to the apical end, all the transition stages between the typical Monocotyledon root to the aberrant root of the adult palm. The succession of these structures depends on growth phenomena.

III. These structures result from the functional activity of the meristematic tissue of the root, passing through a primary stage of tissue development. They appear at the outset with their peculiar structural features at the moment of the formation of the root. They are closely related to the height of insertion in the stem. In proportion to the growth and development of the stem, the meristematic tissues of the roots, which appear at different levels, participate in the stem ontogenetic development.

IV. We know already that a close relation exists between the structures of branches, leaves, and inflorescences, and the stem. A. The leaves arranged along the length of the stem of any annual plant from the base to the tip show the following arrangement: a. Cotyledonary leaves; b. primordial leaves, relatively simple; c. leaves that from node to node show progressive stages of complication until they achieve the typical form of the species considered; d. leaves situated beneath the inflorescences, which are reduced in size, and take the form of bracts.

B. The branches, arising from the development of the axillary buds, have a structure the pattern of which is influenced by that of the stem at the point of their insertion. One knows that the same meristem has formed stems and buds at a given moment; the stem continues to grow while the buds often remain in the resting stage until favorable circumstances arise to release their activity, in the formation of lateral branches. These lateral branches are at the beginning, larger and more complicated in structure when they are inserted on a part of the stem having a higher degree of differentiation and a wider diameter.

C. The inflorescences may be considered from the same point of view. The observations of GRAVIS[11] showed already a correlation between the region of the stem at which the inflorescences appear and the type of structure evolved at this level.

D. The stems themselves show modifications of their anatomy. According to the level, the number of fibro-vascular bundles varies, and so does their arrangement and their pathway through the node as well as the internode. The author previously quoted, studied the different types of

[11] GRAVIS, A. *Urtica dioica* L. Mémoires Acad. Roy. Sci. Belgique. 1896.

structure in the internodes of *Urtica dioica* and *Tradescantia virginica*. At the beginning of the growing period of a stem, the structure becomes successively more complicated, next it becomes uniform and finally undergoes a period of degradation or retrogression.

The meristem at the different nodes of a stem therefore did not function in a uniform manner. It has led to the formation of structures, varying around the specific type, according to the growth periods. These different modifications have for a long time attracted the attention of different workers; they have been attributed to factors of nutrition, to the influence of the surrounding medium. These factors certainly play a part, nevertheless it appears to us that it would be a mistake not to consider the evolution of the growth itself. It is generally said, that the meristem, for example that of a stem, is the youngest and the most active tissue of this stem. Since it is the generative tissue, it is as a matter of course the most active. But, borne at the tip of the stem, during growth, it functions since germination, and in the adult plant, at the extremity of the stem which it forms, it is the oldest tissue present. And this tissue will become still older, the more the stem will elongate over a long period. Therefore, even though it is a generative tissue, the meristem ages. A part of the organographic and anatomical modifications of plants appear to be attributable to this evolution of growth. The vigor of the meristem and the complication of the structures formed are on a par; they augment in proportion as the plant grows up to a maximum, after which there is a slowing down to winter rest or death. This illustrates the bell-shaped curve of the majority of the phenomena of development.

Conclusion

Our observations on the "stilt" roots of the palm, *Iriartea exorrhiza* Mart. make it possible to add a new element to these connections between the morphology and the physiology of development. They establish between *two vegetative organs, roots* and *stems,* anatomical correlations which appear to be due in part to the ontogenetic evolution of the plant. Under the conditions stated for *Iriartea,* where both these organs are formed at the same time from connected meristems, we offer the suggestion, that the parallel variations of structure may be explained by the modifications of the physiological conditions which surround the meristem in the course of development.

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THE INFLUENCE OF ONE ION ON THE ACCUMULATION OF ANOTHER BY PLANT CELLS WITH SPECIAL REFERENCE TO EXPERIMENTS WITH *NITELLA**

D. R. HOAGLAND, A. R. DAVIS, AND P. L. HIBBARD

(WITH FOUR FIGURES)

We have previously published the results of experiments on *Nitella* cells, which emphasized the primary importance of illumination and of temperature in determining the accumulation[1] of halogens in the cell sap and at the same time a number of preliminary observations were reported with regard to certain inter-ionic effects in relation to the process of accumulation (**5**). This latter question had also been studied earlier in connection with experiments on the absorption of ions by barley plants (**4**). While it appears that the accumulation of solutes by a plant cell is dependent on the growth or metabolic activities of the cell, it is also clear that the magnitude of the actual accumulation under any given conditions of light and temperature is influenced by the concentration and composition of the culture solution. It is this latter aspect of the question that we now wish to discuss.

The general methods of experimentation heretofore described were used in the present work. *Nitella* cells were immersed in the solutions under investigation and at the end of the experiments the cells were thoroughly rinsed with distilled water and the larger cells (from 1–3 inches in length) were broken individually, the sap being collected in amounts varying between 1 and 25 cc. Since a single cell yielded only one or a few hundredths of a cubic centimeter of sap, each composite sample of sap collected represented a very large number of cells.[2] The method of HIBBARD (**3**), specially developed for this purpose, was employed in analyzing the sap for its halogen content, which procedure made it possible to obtain results of sufficient accuracy even when dealing with very small volumes of sap. (Determinations were made in duplicate or triplicate.)

* Presented before the American Society of Plant Physiologists, Nashville, December, 1927.

[1] This term is used to indicate the process of concentrating solutes in the cell sap, with the attainment of a higher concentration inside than outside, as in the sense of OSTERHOUT.

[2] It is to be noted that different lots of cells may differ in their physiological state, so that quantitative comparisons must usually be made within each experiment.

Since the earlier experiments had shown that the accumulation of halogens in the cell sap is dependent upon adequate illumination and that the process goes on relatively slowly in any case, we have conducted our preesnt experiments with reference to these conditions. In certain instances artificial light of considerable intensity was employed; sometimes the illumination was continuous. The exposure times extended in some instances to two weeks or more, a point especially to be noted, since it indicates that we were dealing with physiological processes involved in mineral nutrition, and not simply with degrees of permeability. When sufficient time is permitted to elapse a perfectly definite accumulation in the cell sap of certain mineral elements, sometimes of relatively large magnitude, can be demonstrated. If a very limited period of time were employed with similar solutions, it might appear that the cells were almost impermeable to electrolytes and thus the processes most vital to nutrition might escape detection.

The relation of concentration to accumulation

The first question to be considered is concerned with the relation between ionic concentrations in the culture medium and in the cell sap. This question was studied by determining how variations in the concentrations of Br ions in the culture medium influenced the accumulation of these ions in the cell sap. Several experiments were performed. In one experiment the accumulation was permitted to continue for one day only, while in another experiment the cells were kept in contact with the bromide containing solutions for over a month. The results of three different experiments are plotted in fig. 1, in which the curves are of a logarithmic type. Thus the factor obtained by dividing internal by external concentration has a much higher value for external solutions of low concentration than for those of high concentration. Special significance is attached to the present data, inasmuch as they reflect directly the conditions existing in the cell sap, but it is interesting and important to recognize that these experiments on *Nitella* cells are entirely consistent with those made on other plants by necessarily less satisfactory methods. Our own results using agricultural plants give evidence of the existence of similar relations between internal and external concentrations of electrolytes; and likewise consistent are the results on storage tissues reported by Stiles and Kidd (9). All of the investigations emphasize a principle which is highly important to the student of soils and plants, for the reason that in general the concentrations of essential elements in soil solutions are low, sometimes extremely low, and it is essential that land plants should possess a means of concentrating inorganic elements at a relatively high rate from solutions of low concentration.

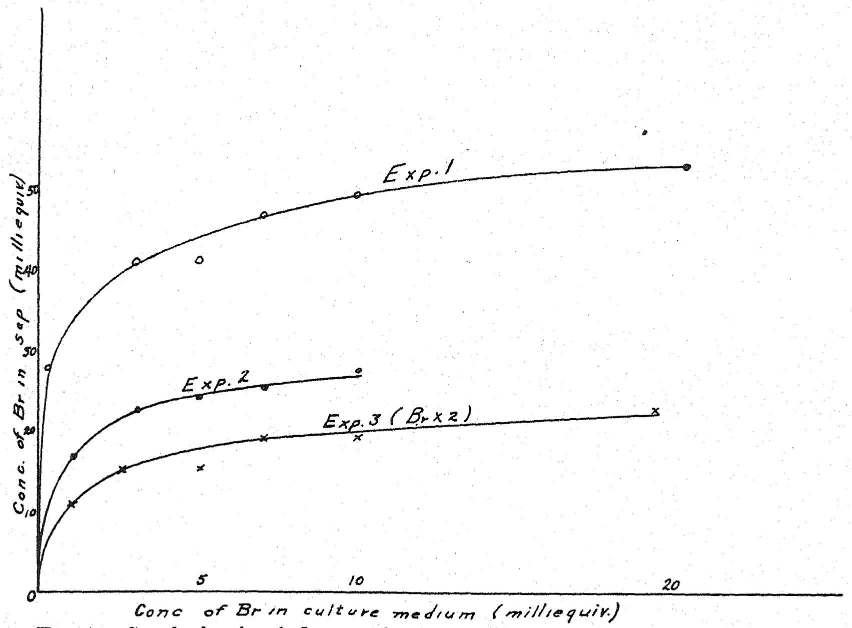

FIG. 1. Graph showing influence of concentration of Br in solution on accumulation of sap. Exp. 1, 9 days' duration. Continuous artificial illumination from two 300-watt lamps; temperature 23°–25° C. Exp. 2, 13 days' duration; diffuse daylight; room temperature. Exp. 3, 27 hours' duration; diffuse daylight plus continuous artificial illumination, two 300-watt lamps. Experiments 1 and 3, without buffer. Exp. 2 with phosphate buffer. Initial pH of all solutions, 5.0–5.4.

If so desired, the data under discussion could be fitted into one of the well known adsorption formulae, but it has not appealed to us that this method of interpretation assists very materially in explaining the mechanism involved, especially when we bear in mind the importance of the metabolic activities of the plant in connection with the ability to concentrate solutes in the interior of cells, as well as the evidence in support of the idea that the chemical elements in question exist in the cell sap primarily in dissociated form, and not for the most part adsorbed on organic compounds. Furthermore, the adsorption formula is of too general a character to serve as a guide to the understanding of such a highly complex series of physiological processes.

Effect of cations and anions on accumulation of Br ions

As another phase of the research we planned to determine to what extent the accumulation of Br ions could be modified by other ions of the same or opposite charge present in the culture solution. First referring to the influence of other anions, experiments were carried out with solutions

containing KBr to which were added KNO_3, KCl, K_2SO_4, or KI in various concentrations (figs. 2–3, tables I–II). The NO_3 and SO_4 ions had no effect whatever in retarding the accumulation of Br ions in the cell sap.[3] In fact the tendency was in the opposite direction, which we attribute to the accelerating influence of the increased K concentration, an effect definitely suggested by experiments to be discussed later in this paper. In marked contrast to NO_3 and SO_4 ions, Cl ions in equivalent concentration signifi-

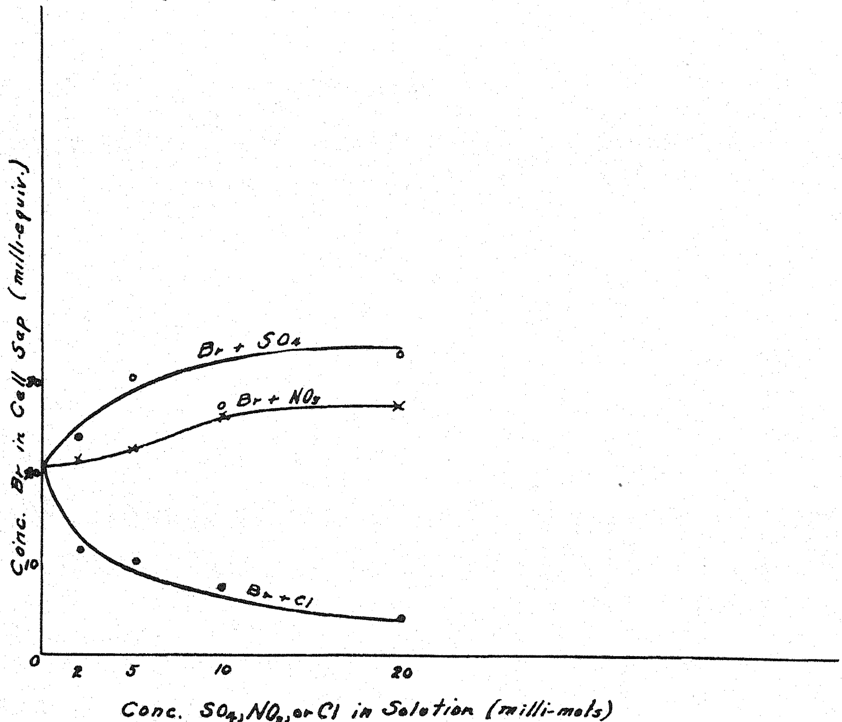

FIG. 2. Graph showing influence of K_2SO_4, KNO_3 and KCl in solution, on accumulation of Br in cell sap; 4 days' duration; continuous artificial illumination with two 300-watt lamps; temperature 23°–24° C. Initial pH of all solutions, approximately 5.0. Phosphate buffer solutions used, containing 0.005 M. KBr to which were added K_2SO_4, or KCl as indicated.

cantly retarded the accumulation of Br in the cell sap and I ions were also definitely effective in the same way, although not to the same degree as Cl ions. A pronounced retardation in the accumulation of Br ions occurred as a result of the presence in the culture solution of Cl ions in a concentration as low as 0.002 molar.

[3] Experiments in which the accumulation of Br from unbuffered solutions and from solutions buffered with phosphate suggest that phosphate ions fall in this respect in the same category with SO_4 or NO_3 ions.

Evidently in these experiments, we have a very clear illustration of one anion influencing the intake and accumulation of another anion. These effects occur in very dilute solutions and with the exception of the higher concentrations of iodide, apparently do not involve injury; therefore it is doubtful whether we should refer to them as phenomena of antagonism. Naturally this depends entirely on the definition one may desire to apply to

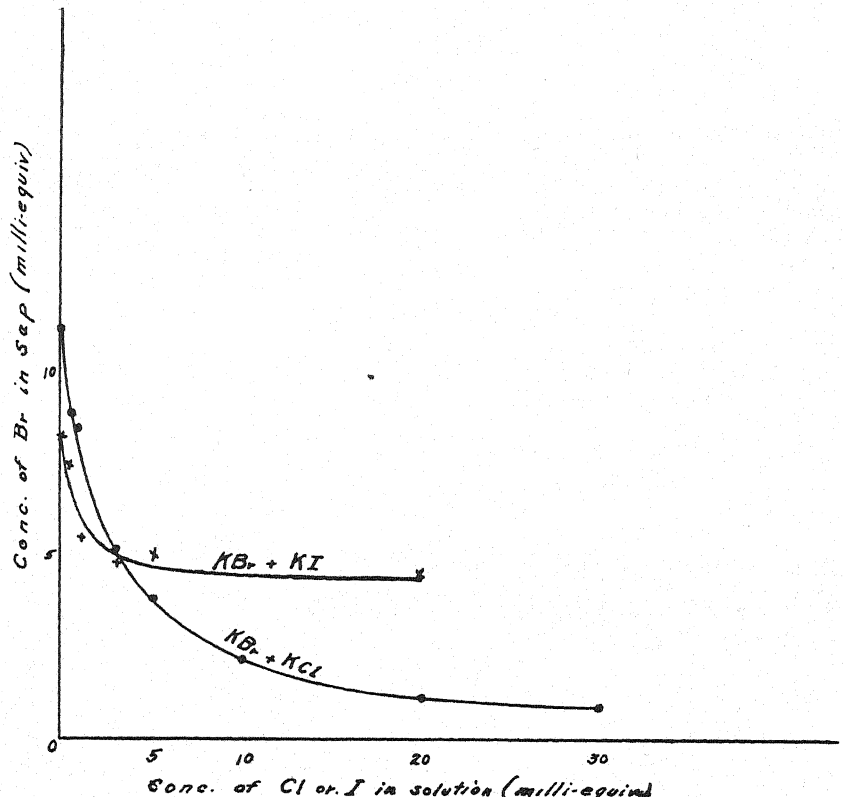

FIG. 3. Graph showing influence of KI and KCl in solution on accumulation of Br in cell sap. Exp. 1, 0.001 M. KBr solutions to which were added KI as indicated. Five days' duration; diffuse daylight plus continuous artificial illumination from two 300-watt lamps. Temperature, 20°–25° C. Initial pH, 5.6–5.8. Final pH, 5.8–6.2. Exp. 2, 0.001 M. KBr solutions to which were added KCl as indicated. Nine days' duration; illumination as in exp. 1; temperature 20°–25° C.; pH values similar to those of exp. 1.

the term "antagonism." When the cells were exposed to certain solutions of iodides during a period of ten days or more, a definite toxicity was observed in several of the experiments, whether caused directly by the accumulation of I ions, or by the formation of molecular iodine, subsequently. The presence of Br ions delayed or inhibited such toxicity. Pos-

sibly this might be considered as an example of antagonism occurring in dilute solution and becoming manifest only after a considerable interval of time.

TABLE I

EFFECT OF OTHER ANIONS ON ACCUMULATION OF BR IONS IN CELL SAP (BR PRESENT IN ALL SOLUTIONS AS 0.005 M. KBR)

CONDITIONS OF EXPOSURE	CONCENTRATION OF ADDED SALT (MOLAL)	CONCENTRATION OF BR IN SAP (MILLIEQUIVALENT)
	Experiment I	
Diffuse daylight, 10 day period, phosphate buffer solution* used. Room temperature.	none	15.2
	K_2SO_4 0.005	19.3
	KCl 0.005	7.3
	KI 0.005	8.2
	Experiment II	
Diffuse daylight, 12 day period, phosphate buffer solutions* used. Initial pH 5.0, final 5.8–5.9. Room temperature.	none	32.0
	K_2SO_4 0.005	35.0
	KNO_3 0.005	31.8
	KCl 0.005	13.8
	KI 0.005	15.5

* General buffer solution
0.002 M. $Ca(H_2PO_4)_2$
0.005 M. KH_2PO_4
Initial PH of such solutions usually between 5.0 and 5.4 and final pH between 5.6 and 6.0.

The results obtained with Br and I give an opportunity for relating potential rapidity of accumulation and reciprocal ion effects. It appears that anions which themselves are incapable of rapid accumulation are also incapable of having a marked influence on the accumulation of other anions. For example, sulphate ions, which are capable of only slow accumulation, do not retard the accumulation of Br appreciably. While it is possible for I ions to accumulate in the cell sap in considerable concentration, several experiments indicate that the accumulation of Br ions may be decreased through the presence of I ions in the solution, without the latter ions being accumulated in the sap in an amount equivalent to the decrease of Br ions. Obviously there exists a reciprocal relation between Br and I ions, so that the sum of the equivalents of halogens accumulated from the single salt solutions may be much larger than the corresponding value for the mixed solution. This means, of course, a mutual hindrance to penetration into

TABLE II

RELATIONS BETWEEN BR AND I IN ACCUMULATION

CONDITIONS OF EXPOSURE	BR IN SOLUTION (INITIAL) MILLI-EQUIV.	I IN SOLUTION (INITIAL) MILLI-EQUIV.	BR IN SAP MILLI-EQUIV.	I IN SAP MILLI-EQUIV.
Experiment I†				
Diffuse daylight and artificial illumination (2–300-watt lamps) continuous illumination for 6 days. Total period 10 days.	5.0	......	58.6	....
	......	5.0	....	19.9
	5.0	5.0	32.6	1.5
Experiment II†				
In light chamber.* 1,800 watts continuous illumination. Period 10 days.	5.0	......	55.5	....
	......	5.0	....	30.1
	5.0	5.0	41.5	6.8
Experiment III†				
Diffuse daylight and artificial illumination (2–300-watt lamps) 10 days, and 5 days in light chamber,* 3,000 watts. Continuous illumination throughout.	5.0	......	61.7	......
	5.0	5.0	46.6	4.0
Experiment IV†				
Diffuse daylight and artificial illumination (2–300-watt lamps) continuous illumination, 11-day period.	5.0	......	22.0	......
	......	5.0	....	6.0
	5.0	5.0	24.7	3.1

* A glass chamber 56 in. × 26 in. with lights evenly distributed outside chamber about 1 ft. from glass.

† Experiments I and II used phosphate buffer solution (table I) pH values 5.4–6.0. Experiments III and IV no buffer used. Similar pH values. Temperature in experiments not controlled, but varied between 20–25° C. Comparisons to be made within each individual experiment. In experiment II one lot of cells immersed in KI solution were too severely injured to be used. In experiment III nearly all cells in KI solution were killed. Cells in KI plus KBr solution in good condition.

the sap occurring somewhere in the protoplasmic layer. Apparently anions which do not have the potentiality of rapid accumulation in the sap are also incapable of penetrating the protoplasm to such an extent as to interfere with the accumulation of other ions. In comparing Br and I it will be observed that Br had a much greater relative effect on the accumulation

of I than I had on the accumulation of Br, and that Br could be accumulated by the cell more rapidly than I. If we can compare these results with the well known antagonistic effects between cations it is clear that anions may "antagonize" each other in an equally significant way, even in very dilute solutions.

We shall discuss next the possible influence of cations on the accumulation of anions. To test this possibility, experiments were made with solutions of various bromides and the accumulation of Br ions in the cell sap determined. It is clear from fig. 4 that the type of cation employed did

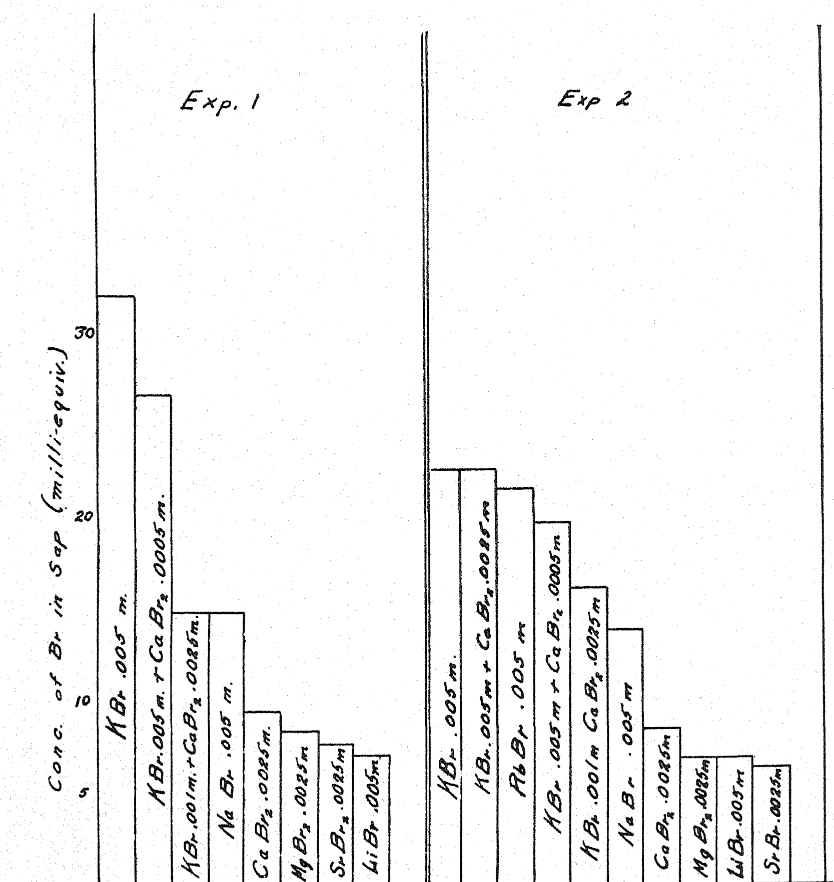

FIG. 4. Graphic representation of accumulation of Br in cell sap from solutions of various bromides. Exp. 1, three days' duration; daylight supplemented by artificial illumination at night from two 300-watt lamps; room temperature (20°–25° C.). Exp. 2, two days' duration; daylight plus continuous artificial illumination from two 300-watt lamps; room temperature (20°–25° C.). Initial pH of all solutions similar (5.6–5.8).

have a marked influence on the concentration of Br attained in the sap after a given exposure to the bromide solutions. The cations fall in three general groups, K and Rb; Na; Ca, Mg, Sr, Li, in the order of decreasing Br accumulation. These general relations were entirely consistent in two experiments, using different periods of time and conditions of exposure. Duplicate determinations of Br were made on each sample of sap with excellent agreement.

In several instances mixtures of KBr and $CaBr_2$ were used. In these cases (fig. 4) K had the dominant influence so that considerable excess of Ca had to be present in order to lower markedly the accumulation of Br from KBr solutions. These relations are not entirely similar to those ordinarily met with in experiments on antagonism, in which very small proportions of Ca may cause great changes in the physiological properties of a solution. In the present study the rate of accumulation of the anion was conditioned primarily by the presence in suitable concentration of a cation capable of ready penetration and accumulation rather than upon alterations in the protoplasm resulting from different proportions of mono- and di-valent ions. Probably a distinction should be made between cation-anion relations and those just discussed with reference to anions. The cation-anion relation may be electrostatic in nature and occur at outer surfaces.

In discussing the cation-anion relations it is suggestive to recall an experiment in which *Nitella* cells were allowed to accumulate Cl from a KCl solution to a concentration significantly greater than that normally existing in the cell sap. When these cells containing the increased amount of Cl were placed in a KBr solution they did not accumulate Br nearly so rapidly as similar cells taken from tap water. The Br which was accumulated by the former cells was accounted for by a nearly equivalent displacement of Cl from the sap, whereas the cells with the normal Cl content accumulated Br as a result both of exchange of Br for Cl and of simultaneous intake of K and Br. With the information now at hand these results can be explained on the assumption that the accumulation of Cl in the preliminary period was accompanied by an accumulation of K to a point approaching equilibrium, so that in the subsequent period of exposure to a KBr solution the cells could not readily accumulate an additional amount of K, thus restricting the Br accumulation to that represented by an exchange of Cl and Br. If this reasoning be correct we have another interesting example of the influence of the accumulation of the cation on the accumulation of the anion.

Since cation-anion and anion-anion interrelations exist it might be anticipated that one cation could influence the accumulation of another cation in dilute solutions, and we believe this to be true. However, it is not prac-

ticable at present to obtain satisfactory quantitative evidence with *Nitella* cells. The most rapidly accumulated cations, K and Rb, are exceedingly difficult to separate by any analytical method, while Cs is probably toxic. In experiments with plants of the agricultural type, in which accumulation of most ions proceeds sufficiently rapidly, it is possible to obtain very definite evidence of inter-ionic relations between cations in dilute solutions of the ordinary type.

It does not follow from the foregoing statements that large accumulations of Br ions may not take place from solutions of salts, the cations of which accumulate very slowly, for the contrary is true. Under such circumstances, Br ions penetrate into the cell chiefly in exchange for Cl ions, and the latter enter the outside solution, as has been pointed out in a previous paper. (When Br is accumulated from KBr solutions, simultaneous accumulation of K and Br is involved as well as exchange between Cl and Br.) Furthermore there is the possibility that ions may enter the cell to some slight extent in conjunction with H or OH ions, although the available data do not indicate that this method of accumulation is of primary importance in the experiments under discussion.

The influence of hydrogen-ion concentration on the accumulation of Br ions

The rôle of hydrogen-ion concentration in the accumulation of other ions is undoubtedly a very complicated one. When we used masses of very small young cells and examined the culture solution, instead of the cells themselves, we found that the removal of Br or Cl ions was much more pronounced from an acid solution (pH 5–6) than from an alkaline one (pH 8–9). A similar effect was shown for the entrance of NO_3 into large cells by direct examination of the cell sap of individual cells. We have not been able to show, however, equally marked effects of hydrogen-ion concentration on the accumulation of Br by large cells, although the indications are that a pH of 6–7 is most favorable to the accumulation. It may be noted that M. M. Brooks (1) in studying the penetration of methylene blue into *Vallonia* cells finds that the rate and not the equilibrium value is influenced by reaction. Our results do not necessarily give an accurate idea of rates.

It is certain that large accumulations of Br ions may take place at any of the pH values experimented with, namely, pH 5–8 (table III). In passing, attention is called to the observation that Br ions may become much more concentrated in the cell sap than in the culture solution even when the pH value of the latter is the same as that of the cell sap, so that no gradient of H or OH ions is necessarily required for the process of accumulation. This fact may be of interest in connection with hypotheses concern-

TABLE III

EFFECT OF HYDROGEN-ION CONCENTRATION ON ACCUMULATION OF BR

CONDITIONS OF EXPERIMENT	INITIAL pH	FINAL pH	CONCENTRATION OF BR IN SAP MILLIEQUIV.
Experiment I*			
Diffuse daylight plus continuous artificial illumination (2–300-watt lamps), 5-day period. Room temperature.	5.4	5.6	20.7
	6.4	6.8	31.7
	8.2	7.6	20.6
Experiment II*			
Light chamber, 3,000 watts continuous illumination, 3-day period. Temperature 20–25° C.	5.4	......	30.6
	6.4	......	35.0
	7.6	......	27.9
Experiment III*			
Diffuse daylight, 3-day period. Room temperature.	5.5	5.9	7.4
	6.4	6.6	17.7
	7.4	7.4	16.9
	8.6	7.4	12.7
Experiment IV*			
Diffuse daylight, 5-day period. Room temperature.	5.2	6.0	15.6
	6.0	6.6	31.0
	7.0	7.2	34.4
	8.0	7.9	27.8
	8.3	7.9	19.5
Experiment V*			
Continuous artificial illumination (2–300-watt lamps), 3-day period. Average temperature, 24° C.	4.9	5.5	19.3
	6.0	6.0	24.4
	6.9	6.9	26.4
	7.8	7.8	30.4

* KH_2PO_4 plus NaOH used to make buffer solution. Experiments I, II, III, KH_2PO_4 0.001 M. Experiments IV, V, K_2PO_4 0.0025 M. KBr 0.005 M. present in all solutions.

ing the mechanism of accumulation. It is also worthy of comment that the accumulation of both cations and anions takes place on the alkaline side of the average isoelectric point of the proteins present in the cell, as determined by PEARSALL (8). There does not seem to be available any explana-

tion of ion accumulation on a simple basis of ion protein combinations. It would be difficult to assume that individual proteins are available of such widely different isoelectric points as to permit the simultaneous formation of both cation and anion compounds at the pH values involved.[4]

General discussion

It will be remarked that we have explained all of our data in terms of ions. We are, of course, aware that the hypothesis has been advanced that only undissociated molecules are able to penetrate into living cells. Certain experiments with weak acids and with dyes have been made which lend support to this hypothesis (**6**, **7**). (Some work by M. M. Brooks (**1**) on dyes tends rather to an interpretation in terms of ions.) Concerning these data we do not know to what extent they bear on the results we have presented. As we have suggested previously, in the systems containing the weak acids employed, gaseous components were present and it is not certain that they can be disregarded. In the solutions with which we have experimented, the salts were possibly completely dissociated according to present theories, and in the sap the salts also must have existed primarily in dissociated form for reasons discussed elsewhere. It would require very complicated assumptions to explain our results on any other than an ionic basis. For example, we have to consider the unequal accumulation of the ions of a salt such as $CaBr_2$, the Br accumulation in this case being accompanied by a marked loss of Cl from the cell. An explanation is required also for the very different effects of different anions on the accumulation of Br. In fact, all of the observations seem to be most easily understood by assuming that ionic processes are involved. It is true, however, that the actual mechanism of accumulation is as yet unsolved, so that it would be unwarranted to make positive statements, especially as the physical chemistry of even simple ionic systems appears to be in a stage of further development.

Although the data we have discussed in this paper are confined to one algal organism, we feel that the general relations which have been shown to exist between the cell sap and the culture medium apply to a wide range of plants. Indeed our experiments on agricultural plants are consistent with this statement. With regard to the comparative rates of accumulation of specific ions it would seem that this question is not wholly one of ionic properties, but involves the type of plant metabolism as well. For example, many agricultural plants are capable of very rapid accumulation of nitrate,

[4] Note, however, recent article by G. E. Briggs and A. H. K. Petrie, Biochemical Jour., Vol. XXII, No. 4, pp. 1071–1082 (1928) also J. Davidson, Jour. Agr. Res. Vol. 35, No. 4, pp. 335–346 (1927).

but this does not seem to be true of *Nitella* cells. If we consider the cation relations, as they are reflected by the present experiments, the physiological order would be consistent in a general way with the mobilities of the ions, but with the anions the matter is in doubt. A complex ion like NO_3 or SO_4, capable also of reduction, is very difficult to place on a purely electrochemical basis.[5] Comparing the results on other plant cells, obtained by STILES and KIDD (**10**) and by several other investigators, there is a general agreement as to the order of the ions, with the exception of the NO_3 ion, as already mentioned. Recently, COOPER and WILSON (**2**) have suggested that the electromotive force is the most important characteristic of the ion to be considered, but the details of their researches are as yet unavailable.

Summary

1. Further experiments on *Nitella* cells are reported pertaining to the accumulation of halogens in the cell sap. Analyses were made of composite samples of sap obtained from individual cells. Consideration was given to the maintenance of conditions of illumination essential to the process of accumulation.

2. The relation between Br ion concentrations in the culture medium and in the cell sap was of a logarithmic type. This is in agreement with results obtained on other plant cells using other electrolytes.

3. The accumulation of Br ions was significantly retarded by Cl or I ions also present in the culture medium, but not by SO_4 NO_3 or PO_4 ions. These and other ionic effects discussed occurred in very dilute solutions. Consideration is given to the relation between the retarding effects of one ion on another and potentiality of accumulation in the sap.

4. The accumulation of Br ions was definitely influenced by the nature of the cation, being most rapid when solutions of KBr or RbBr were used, and least rapid with solutions of LiBr, $CaBr_2$, $SrBr_2$ and $MgBr_2$.

5. With regard to the effects of hydrogen-ion concentration, marked accumulation of Br ions took place at reactions varying between pH 5 and 8.

6. Taking all the data into consideration it is found very difficult to explain them on any other than an ionic basis.

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THE PREPARATION OF CHLOROPHYLL*

F. M. SCHERTZ

Introduction

This paper is published in response to repeated requests for information regarding the preparation of pure chlorophyll. It also shows the relative purity of the samples of chlorophyll which have been used as the basis of investigations conducted in this laboratory. It is hoped that the method here described will be helpful to those contemplating a study of this important pigment. Chlorophyll is so important that it is felt that every effort should be put forth by workers to obtain a product of undoubted purity. An effort has been made to present the details of the preparation so that the difficult parts may be mastered by most investigators after a little preliminary work on their part.

Material used

The choice of material to be used in the preparation of chlorophyll is very important for in the leaves of many species of plants chlorophyll decomposes during the process of drying. This is true of leaves of the following plants: alfalfa, spinach, cowpea, and many others. In fact very few leaves will dry entirely satisfactorily. Consequently, in order to save much valuable time and material it is best to use the leaves of only such species as have proven entirely satisfactory. Leaves of *Urtica dioica* have been shown by experience to be best, while those of *Urticastrum divaricata* are very good.

The leaves for our preparation were collected during the active growing season (April or May) and were then spread out on screens to dry, away from direct sunlight. A temperature of 30–40° C. was used for drying. Twenty-four hours are usually sufficient. Electric fans and hot plates were used to hasten the drying process. As soon as the leaves were dry enough to crumble easily in the hands, they were ground finely in a pebble mill. The material should not be ground too finely otherwise the extraction will be more difficult. The method of extraction and purification as given here is the method which has been found to remove the impurities most satisfactorily. All of the operations in the preparation of chlorophyll should be carried out as quickly as possible.

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Method of extraction and purification

Two moistened filter-papers are placed in a 25-cm. suction funnel (this size of funnel is used because the layer of meal should not be more than 4–5 cm. thick), and sucked down firmly. One kg. of the leaf meal is loosely placed into the funnel and 2 liters of 80 per cent. acetone (by volume) are poured on and allowed to sink into the meal till some of the extract runs from the funnel below. Suction is applied and the extract is sucked off into a filter-flask. Suction at no time should make the acetone extract boil or the filtration will be hindered. More acetone (1 liter of 80 per cent.) is added and sucked off. This is repeated till all of the green pigment is extracted from the leaf meal. About 3–6 liters of the 80 per cent. acetone are required for the extraction. Finally, the decolorized leaf meal is sucked dry to remove all of the acetone extract from the leaf powder.

The vivid green extract is transferred into 1.5 l. of petroleum ether (B. P. 30–70° C.) in a 6-liter separatory funnel (a). The petroleum ether will take up the four chloroplast pigments and a weakly yellowish green aqueous acetone layer will separate below. This aqueous acetone layer is run in a thin stream through a 4-l. separatory funnel (b) which contains 1 l. of petroleum ether. The petroleum ether in this funnel will remove practically all of the chloroplast pigments. The aqueous acetone from which the chloroplast pigments have been separated is poured into a 6-liter separatory funnel (c) which contains 0.5 l. of petroleum ether. This last separatory funnel of petroleum ether is to break emulsions which will form in the process described in this paper and to remove any traces of chlorophyll which may have passed through the other funnels.

The petroleum ether in the three funnels is now washed with a liter of 80 per cent. acetone which removes impurities and takes away none of the chlorophyll. The washing is carried out by allowing the acetone to run in a fine stream through the petroleum ether solutions. The petroleum ether by taking up acetone has increased considerably in volume. The acetone is removed by allowing a liter of distilled water to flow into *a* and then on down through *b* from a liter separatory funnel (*d*) above *a*. By the above method, much of the accompanying substances are removed, the greater per cent. of the acetone is separated and emulsions are prevented.

The solutions are never shaken at any time throughout this procedure, for emulsions once formed are difficult to break and this method prevents their formation to any great extent. All of the wash liquors from *b* are passed through funnel *c* from which they are run off. A large per cent. of the acetone may be recovered from the aqueous acetone by distillation, and used again.

The acetone is not quantitatively washed from the petroleum ether solutions, for the chlorophylls and xanthophyll would precipitate, and the purification would be made much more difficult; for then the xanthophyll could not be separated from the petroleum ether by the methyl alcohol washings.

The xanthophyll is now separated from the chlorophylls and carotin by allowing 4 liters or more of 80 per cent. (by volume) of methyl alcohol to run down in a fine stream through the petroleum ether in the separatory funnels. The last liter of methyl alcohol may be cautiously shaken with the petroleum ether solution of chlorophyll. The methyl alcoholic extract of xanthophyll is run out of each separatory funnel into the next as rapidly as it separates from the petroleum ether layer, for in this way more of the xanthophyll will be removed from the petroleum ether layers and a purer chlorophyll product will result. The methyl alcoholic extracts may be worked up for xanthophyll by the methods described by WILLSTÄTTER and STOLL.

The last traces of methyl alcohol and acetone are now removed by allowing a fine stream of water to run from separatory funnel *d* down through the petroleum ether solutions. In all, 8 to 12 liters of distilled water are required for this washing. The petroleum ether gradually loses its fluorescence, becomes turbid and the chlorophylls precipitate. Toward the end of the washing, emulsions will form but these may be broken by dissolving salt (NaCl) in the wash water from funnel *b* and then the saline solution is poured into separatory funnel *c*, where most of the emulsion will disappear. If the emulsion still persists it may be broken by running off the aqueous layer from funnel *c* and adding more salt. The saturated solution is returned to funnel *c* and this process is repeated till most of the water is removed from the petroleum ether layer.

The petroleum ether suspension of chlorophyll is now shaken with about 250 gm. of anhydrous Na_2SO_4 which removes the last traces of water and makes the solution filterable. The petroleum ether and accompanying impurities are now separated from the chlorophyll by filtering through a layer of talc on a 25 cm. suction funnel. The layer of chlorophyll which forms upon the talc is constantly broken by stirring with a nickel spatula, otherwise filtration would proceed very slowly. The petroleum ether filtrate obtained contains very little chlorophyll, practically all of the carotin and only a very little of the xanthophyll. From this filtrate carotin may be obtained. The suction applied in this filtration should be very moderate else the filtration will be retarded. Finally, wash the mass of chlorophyll and talc with 500 cc. or more of petroleum ether. Then, apply strong suction to remove as much of the petroleum ether as possible.

A. The acetone method of purification

The chlorophyll-containing talc is removed from the suction funnel and placed in a beaker where it is stirred for a short time with pure acetone (500 cc.). The acetone solution of chlorophyll is now filtered from the mass by placing the whole upon a suction funnel (15 cm. in diameter) and washing the talc with acetone till all of the chlorophyll is removed.

The acetone solution of chlorophyll is poured into 1 l. of petroleum ether in separatory funnel *a* and 500 cc. of petroleum ether is added to each of the funnels *b* and *c*. Water is now added to the chlorophyll solution until the acetone layer separates. This layer is run through the petroleum ether in *b* and *c*, after which the acetone may be recovered. The acetone is now all washed from the petroleum ether solutions by washing as above with 8–12 liters of distilled water. The chlorophyll precipitates and is obtained by filtering the dried (with anhydrous Na_2SO_4) petroleum ether solution through talc upon a suction funnel (15 cm. in diameter). The precipitated chlorophyll is washed with 0.5 to 1 l. of petroleum ether. The petroleum ether mother liquor here should run off slightly yellowish at first and finally only faintly green.

The chlorophyll-containing talc is now freed of the petroleum ether by allowing the suction to continue for a few minutes. The material may be allowed to stand over night in this condition. This purification has removed traces of carotin and xanthophyll. The chlorophyll is now purified by the petroleum ether method which removes practically all of the impurities.

B. The petroleum ether method of purification

The chlorophyll-containing talc is now removed from the funnel and placed in a beaker where it is stirred for a short time with alcohol-free ether (500 cc.).[1]

As soon as the chlorophyll has gone into solution it is filtered through a thin layer of talc on a separatory funnel (15 cm.) which is just large enough to hold the mass of material. The talc is washed with ether till all of the chlorophyll is removed.

The ether (1 l.) is now evaporated from the chlorophyll solution until a sirupy mass remains. The evaporation is carried out by placing the container in a hot water bath. The level of the hot water is always kept below the level of the ether in the container. When the volume of the chlorophyll solution is reduced to about 100 cc. the ether is then removed by reduced pressure till the mass is sirupy. About a liter of petroleum ether is added a little at a time and the flask is shaken violently till the chlorophyll precipitates.

[1] The ether used here is prepared by washing U.S.P. ether with water several times and then distilling over $CaCl_2$.

The precipitated chlorophyll is now removed from the mother liquor by filtering upon talc and is then washed with about 500 cc. of petroleum ether. It is extracted again with 500 cc. of purified ether and the solution is concentrated at 30–40° C. to a sirupy mass which is then dried in a beaker in a vacuum desiccator. When dry the steel blue shiny mass of chlorophyll may be easily pulverized and weighed.

TABLE I

YIELD OF CHLOROPHYLL ($\alpha + \beta$) OBTAINED FROM LEAVES FROM DIFFERENT SOURCES

SOURCE	METHOD OF PURIFICATION	YIELD IN GM. PER KGM. OF DRY LEAVES
Minnesota, 1924	Ether	2.0
"	Ether	1.4
Missouri, 1924	Ether	6.9
"	Ether	7.1
"	Ether	8.3
"	Ether	8.0
"	Ether	7.9
"	Ether	8.4
"	Ether	8.2
"	Acetone	7.1
Washington, D. C., 1924	Acetone	4.5
"	Acetone	4.3
"	Acetone	3.8
"	Acetone	5.6

Purification of chlorophyll by the methods described here has given the writer his purest product. Consequently, it is submitted as a method of preparing very pure chlorophyll.

Results of extraction by the above methods

Samples of stinging nettle leaves were obtained from Minnesota for extraction and the amount of chlorophyll obtained per kg. from them is compared with that from stinging nettle leaves collected at Washington, D. C. Chlorophyll was also extracted from the leaves of wood nettles from Missouri.[2]

The amount of chlorophyll obtained per kg. of the dried leaves is recorded in table I.

The yields of chlorophyll as given in this table were not all obtained by the method which has just been described above. The first nine were pre-

[2] The author is greatly indebted to Dr. CARL G. DEUBER, formerly of the University of Missouri, now of Yale University, for collecting and drying these leaves.

pared by the method given by WILLSTÄTTER and STOLL and the remainder were prepared by the same method except that they were purified by the acetone method instead of being precipitated by petroleum ether as given by WILLSTÄTTER and STOLL in their monograph on chlorophyll.

The quantity of chlorophyll per kilogram from the leaves from Minnesota was the lowest of all and also the pigment which was obtained was off color when dissolved in ether. The wood nettle leaves obtained from Missouri gave the best results and the chlorophyll obtained was the purest.

Using 0.0513 grams of each sample of chlorophyll[3] (or 0.10 gm. per liter) tests were made for total yellow pigments, and with the colorimeter also to determine which samples contained the least impurities. Those containing the least impurities when saponified gave the lowest readings on the colorimeter. The carotinoids were separated from the chlorophyllins and the results are recorded in table II.

The results shown in table II are for only the samples which were used in the final work on chlorophyll. Many other samples not described or used in this work showed that changes had taken place in the chlorophyll during its extraction and purification, or that the original leaf material was not of the best grade. Samples which did not show a pure green color when dissolved in ether or which failed to give a good phase test were not used.

In the measurements of chlorophyll in columns 3 and 7 of table II, the readings are in millimeters, and the comparisons are made with the following combination of Lovibond slides, nos. 3, 4, and 5 blue, plus 10 and 20 yellow, using a Duboscq colorimeter. The concentration of chlorophyll in the solutions measured is 0.1000 gm. per liter.

The carotinoids, shown in columns 4 and 8 of table II, were estimated colorimetrically as carotin. The amounts given were the amounts found in each 0.05 gm. sample of chlorophyll.[4]

The readings for samples no. 4 and no. 6 show that most of the carotinoids were removed by the acetone method of preparation, as can be seen by the figures in column 3, table II. All of the samples given in column 3 were then purified by the acetone method as given in this paper. Examination of the figures in the last four columns of table II shows that the method was not only valuable in removing the carotinoids but the purity of the chlorophyll samples was greatly improved. (*Cf.* columns 3 and 7 for chlorophyllin content.)

[3] The samples of chlorophyll used here were dried in a vacuum desiccator for two weeks.

[4] SCHERTZ, F. M. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. 26: 383–400. 1923.

TABLE II

PURITY OF CHLOROPHYLL SAMPLES AS SHOWN BY TESTS OF THEIR CAROTINOID AND CHLOROPHYLL CONTENT, AND THE RESULTS OF PURIFICATION BY THE ACETONE METHOD

SAMPLE NUMBER	CHLOROPHYLL YIELD PER KILOGRAM OF DRY LEAVES	CHLOROPHYLLIN CONTENT (COLORIMETER)	CAROTINOID CONTENT	WEIGHT OF CHLOROPHYLL AFTER ACETONE PURIFICATION	LOSS IN PURIFYING	CHLOROPHYLLIN AFTER ACETONE TREATMENT (COLORIMETER)	CAROTINOIDS AFTER ACETONE TREATMENT
	gm.	mm.	mg.	gm.	Per cent.	mm.	mg.
1	8.0	24.9	0.73	5.6	30.0	17.7	0.50
2	8.4	16.5	0.73	6.6	21.0	16.1	0.43
3	8.2	23.2	0.77	6.2	24.0	17.4	0.46
4*	4.3	27.5	0.10	2.9	32.0	16.8	less than 0.10
5	6.9	23.1	0.30	5.7	17.0	15.0	less than 0.10
6*	7.1	24.8	0.10	5.0	29.0	17.8	less than 0.10

* Samples 4 and 6 were prepared by the acetone method while the others were obtained by the ether method.

Further evidence may be submitted in favor of incorporating the acetone method of purification with the method for preparing fairly pure chlorophyll. In all, five samples of chlorophyll were obtained by substituting the acetone method for the ether purification as used by WILLSTÄTTER and STOLL. One of the samples of chlorophyll, when extracted and prepared by the acetone method contained 0.20 mg. of carotinoids while the other four samples contained less than 0.10 mg. per 0.0513 gm. sample of chlorophyll. The average result for fifteen samples obtained by the ether method of WILLSTÄTTER and STOLL was 0.81 mg. of carotinoids per 0.0513 gm. of chlorophyll. The method as modified, then, should be far superior to the method given by WILLSTÄTTER, because not only will the carotinoid content of the samples obtained be less, but the chlorophyll content will be much greater, as shown by the figures in table II.

The samples used here would have been further purified if any method had been available whereby a purer sample could be detected. The error in colorimetric estimation was too great to make any further purification worth while and spectrophotometric methods used with the yellow pigments are not applicable to a solution of mixed pigments. Consequently, these six samples after further testing were regarded as pure chlorophyll, though it is highly probable that they contain a small percentage of impurities.

Before any quantitative experiments could be carried on with the chlorophyll prepared it was necessary to know the loss in weight on heating to 100° C. so that the correct amount of chlorophyll could be weighed out for any given experiment.

Table III shows the weight of the samples of chlorophyll before and after drying in an electric oven over night at 100° C. after the samples had been previously dried in a vacuum desiccator for two weeks. The loss in the samples was generally 0.0007 gm. consequently, 0.0507 grams, which represents 0.0500 gm. of dried chlorophyll, was used in each experiment.[5] In all of the acetone purification experiments in table II, 0.0507 gm. of chlorophyll was used, instead of 0.0513 gm. which was used by WILLSTÄTTER.

The first six samples in table III are those already described in this paper. Samples 7 and 8 in table III were prepared after the ash content of the first six were known. These two samples were prepared by the method described in this paper. The ash content was then determined, the data being given in the last column of table III. These two samples were then again purified by dissolving in ether and precipitating by the addition of petroleum ether. The ash content was then found to be exactly the same

[5] Chlorophyll cannot be dried at 100° C. and then used, for the heat alters the chlorophyll molecule. Chlorophyll after drying is brownish green instead of a pure green when dissolved in ether. If a dried sample is saponified the result is a dirty brownish green chlorophyllin instead of a clear bright green.

TABLE III

LOSS IN WEIGHT BY DRYING CHLOROPHYLL AT 100° C. AFTER DRYING IN A VACUUM DESICCATOR FOR TWO WEEKS OR MORE, AND THE ASH CONTENT OF CHLOROPHYLL PREPARATIONS

SAMPLE NUMBER	WEIGHT BEFORE DRYING	WEIGHT AFTER DRYING	LOSS IN DRYING	ASH CONTENT OF CHLOROPHYLL
	gm.	gm.	gm.	Per cent.
1	0.0513	0.0506	0.0007	3.9
2	0.0519	0.0513	0.0007	4.2
3	0.0520	0.0513	0.0007	3.6
4	0.0514	0.0507	0.0007	3.4
5	0.0520	0.0513	0.0007	4.5
6	0.0507	0.0495	0.0012	4.1
7	....	....	....	4.1
8	....	....	....	4.0

as that already determined. Hence, no further attempts to improve the purity of the chlorophyll were made.

Only one of the samples reported in table III gave the theoretical amount (4.5 per cent.) of ash and this sample was not purified as much as some of the others. No explanation is offered as to why the ash content varies in the samples. It might be suggested that the magnesium of the chlorophyll had been replaced by hydrogen but this was not true, for each of the samples of chlorophyll was carefully tested in the spectroscope for bands due to pheophytin. No trace of pheophytin was found in any of the samples. This test is easy to make for the spectroscopical bands of chlorophyll and of pheophytin are quite different.

The ash of two of the samples were tested for their MgO content. The ash from sample no. 1 was found to yield 100 per cent. MgO while that from no. 5 was found to be 92 per cent. MgO.[6] No other tests to determine the MgO content of the samples were made.

The ash content of samples of chlorophyll prepared by WILLSTÄTTER and his students will now be considered. WILLSTÄTTER and ISLER[7] prepared pure chlorophyll α and β. The amount of ash (MgO) found for the samples of chlorophyll α was 4.3, 4.6, and 4.2 per cent. and for chlorophyll β it was 4.1, 4.4, 4.4, and 4.2 per cent. WILLSTÄTTER and HUG[8] give only 3 results for preparations of pure chlorophyll. They are 4.5, 4.6 and 4.9 per cent.

[6] The author is indebted to R. B. DEEMER of this laboratory for these determinations.

[7] WILLSTÄTTER, R., and ISLER, MAX. Untersuchungen über Chlorophyll. XX. Über die zwei Komponenten des Chlorophylls. Liebig's Annalen. 390: 269–339. 1912.

[8] WILLSTÄTTER, R., and HUG, ERNST. Untersuchungen über Chlorophyll. XV. Isolierung des Chlorophylls. Liebig's Annalen. 380: 177–211. 1911.

Their preliminary results were 3.1, 3.2, 3.9, 3.7, and 3.6 per cent. The pure chlorophyll which WILLSTÄTTER and HUG prepared was obtained by a method which was essentially different than that described in this paper. The leaf meal was given a preliminary washing with benzol and with petroleum ether. The chlorophyll was then extracted with ethyl alcohol, transferred to petroleum ether and then purified. Results of analysis for ash are not given in WILLSTÄTTER and STOLL's monograph on chlorophyll, in which they describe the method which has been modified and described in this paper. Why the results given by WILLSTÄTTER and HUG are higher than the theoretical and why the results given in this paper are lower than the theoretical result, 4.5 per cent., is as yet unexplained.

Tests used in determining purity

PHYTOL CONTENT

Instead of directly determining the phytol content of the chlorophyll, its purity was determined by extracting an ethereal solution of the pigment with 22 per cent. HCl. If the chlorophyll has been altered by the splitting off of the phytol this fact will be indicated because any chlorophyllide present will dissolve in the 22 per cent. HCl. This test is very sensitive.

None of the ethereal solutions of chlorophyll gave any color when extracted with 22 per cent. HCl, hence none of the phytol was split off. If phytol were split off the MgO content would have been too high.

YELLOW PIGMENTS

In the results given in this paper it is seen that none of the chlorophyll preparations was wholly free from yellow pigments, nor has the author succeeded yet in preparing one which is absolutely free from carotinoids.

Most of the preparations here described contained much less than 1 per cent. of the carotinoids; the carotinoid present is xanthophyll.

THE PHASE TEST

The brown phase appeared on saponification with methyl alcoholic potash. Allomerized chlorophyll would not give this test and the solution remains brown with a mixture of pure and allomerized chlorophyll.

THE SPECTRUM

An analysis of the spectrum of the preparations was made to see whether any of the groups in the chlorophyll molecule had been changed in the process of preparation. This observation is far more sensitive in detecting impurities in the sample than would be a chemical analysis of the chlorophyll. If chlorophyll is altered by the action of acid, the presence of pheo-

phytin is observed in the spectrum, for then two absorption bands appear; one before the Fraunhofer line E and the other between the lines E and F. No such pheophytin absorption bands were found in the spectra of these preparations. The absence of these bands is evidence of purity of the samples.

The magnesium complex of all the preparations was found to be unaltered.

Color tests

In order to tell which preparations of chlorophyll were good, their ether extracts were compared as to color. Several samples were compared at one time and only those showing a pure green color were considered good. Traces of allomerization could be easily detected in this manner.

Allomerization was further tested for by saponifying these ethereal solutions of chlorophyll. Good chlorophyll showed a pure, clear green while allomerized chlorophyll was of a dirty green color, after the saponification.

Summary

1. A method is described for the extraction and purification of chlorophyll $(\alpha + \beta)$.

2. From 7–8 grams of chlorophyll may be obtained from 1 kg. of nettle leaves.

3. Purification of chlorophyll by the acetone method described is recommended because by its use most of the carotinoids are removed and the chlorophyll was found to be much purer. The method as outlined is recommended for obtaining very pure chlorophyll. If large yields of chlorophyll, not so pure, are desired, then the method may be easily modified to meet the needs of the worker.

4. 0.0500 gram samples lost about 0.0007 gm. on drying at 100° C.

5. The chlorophyll preparations described here yielded on an average 3.98 per cent. of ash. Two samples of the ash were analyzed and found to contain 92 and 100 per cent. MgO.

6. The tests used in determining the purity of chlorophyll are described.

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A METHOD FOR THE DETERMINATION OF INORGANIC NITROGEN IN PLANT EXTRACTS[1]

A. C. SESSIONS AND J. W. SHIVE

(WITH ONE FIGURE)

Numerous methods have been used for the determination of nitrate nitrogen in plant tissue, but a review of the recent literature shows the Ulsch method (**1**), the Kjeldahl-Gunning-Arnold method to include nitrates (**1**), and the Devarda method (**9**), to be the three most widely used at the present time. All of these methods, however, have been severely criticized.

NIGHTINGALE, ROBBINS and SCHERMERHORN (**4**) called attention to the fact that nitrogen obtained by the Ulsch method includes not only nitrate nitrogen but also any amide nitrogen which may be present. GALLAGHER (**3**) criticizes the Ulsch method, for, as he states, in the reduction of nitrates by means of an acid reducing agent, the first step in the process appears to be the formation of nitrites. In the presence of acid, nitrous acid is formed, and this may react with amino groups which may be present, either in the form of amino acids or proteins, with the liberation of nitrogen. However, if heat is not applied too rapidly, the loss of gaseous nitrogen apparently does not occur (**4**) though the possibility of loss of nitrogen due to improper manipulation is a serious objection to the method, as well as the necessity of working with amide-free solutions. STROWD (**9**) concludes that the determination of nitrate nitrogen in plants by finding the difference between the Kjeldahl method to include nitrates and the Kjeldahl-Gunning-Arnold method is unsatisfactory, since appreciable amounts of nitrates are apparently reduced, even without the addition of reducing agents.

Many workers are now determining the nitrate nitrogen of plant extracts by the Devarda method. In this method, 0.4 to 1.0 gram of sodium hydroxide is added to each 100 cc. of the plant extract, and this alkaline solution is distilled with one to two grams of Devarda's alloy for one hour. The ammonia obtained from the reduction of the nitrates is absorbed in standard acid and titrated against standard alkali. This method was recommended by STROWD (**9**) in 1920, but BURRELL and PHILLIPS (**2**) could not obtain concurrent results when amide nitrogen was present in the extract. Recently RANKER (**8**) has shown that this method as ordinarily used gives values which in no way represent the nitrate nitrogen in biolog-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

ical material. PHILLIPS (5) suggests that this method may be used successfully, provided that the ammonia and amide nitrogen is removed prior to the nitrate determination.

BURRELL and PHILLIPS (2) used the phenoldisulphonic acid method, but failed to obtain consistent results until they had cleared the extract, removed the acid-charred organic matter, and freed the solution from chlorides. Due to the complications which these processes introduce, this colorimetric method is little used.

PYNE (7) has recently developed two methods which appear to give reliable results. The first method is based upon the reducing power of titanous hydroxide in a cold alkaline solution; the second depends upon the reduction of nitrates by Devarda's alloy in the cold, the conversion of the ammonia formed into ammonium chloride, and distillation *in vacuo* at 45°–50° C. with ignited lime.

In the method to be described in the following pages, nitrates are reduced to ammonia by the use of cold eighth-normal alkali and finely ground Devarda's alloy. The ammonia thus formed is recovered by the Folin aspiration method.

The work was carried out in the Laboratory of Plant Physiology of the New Jersey Agricultural Experiment Station, and it is a pleasure to acknowledge indebtedness to Dr. G. T. NIGHTINGALE and to Dr. W. C. RUSSELL for many helpful suggestions and encouragement.

Materials

In the method here described, Merck's analytical reagents were used throughout. Sulphuric acid solution, 0.050 normal and 0.033 normal sodium hydroxide solution were used in all titrations. These were standardized against benzoic acid obtained from the United States Bureau of Standards (sample 39 C). Methyl red was used as an indicator, and all titrations were carried to the complete disappearance of any red tinge.

In obtaining the plant extracts, the fresh tissue was ground in a large mortar with nitrogen-free quartz sand. Water was added to the ground material and the whole was transferred to a large funnel over which had been placed an eighteen-inch square of lawn cloth. The extract was forced through the cloth by moderate wringing by hand. All nitrates could be removed from 150 grams of the plant materials here used by two thorough grindings and repeated extractions with two liters of water, as it was found that small portions of the extracted tissues oven-dried at 70° C. gave only a negative reaction with diphenylamine. The resulting extract was heated to boiling, 3 cc. of 10 per cent. acetic acid was added, and boiling was continued for 2 or 3 minutes. The coagulum formed was removed from the

solution by filtering through a Buchner funnel into which was placed a filter paper and nitrogen-free paper pulp. The coagulum-free filtrate was reduced in volume to 500 cc. by gentle boiling, and 50 cc. aliquots were taken for analysis. At no time was the extract thus prepared allowed to stand more than 12 hours before analyzing; usually the analyses were made immediately after extraction.

Apparatus and method

The absorption tubes and aspiration bottles used consist of large test tubes (2.5 × 25 cm.) and 300 cc. Florence flasks, respectively. Twelve of each constitute a battery, and are so arranged that the same current of air is drawn through the entire system. Figure 1 shows the essential features of the apparatus and its arrangement. Fifty cc. of the plant extract is placed in each of the Florence flasks and 2 cc. of paraffin oil is added.

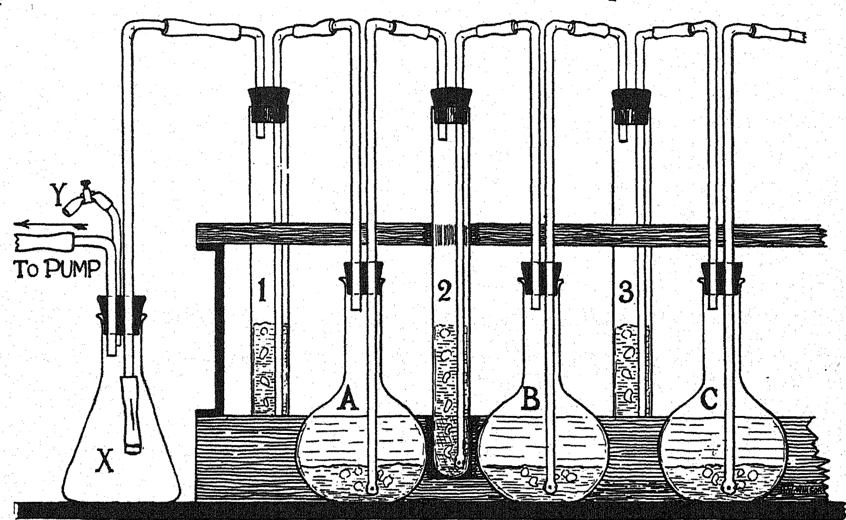

FIG. 1. Aspirating system for determining inorganic nitrogen in plant extracts. Description in text.

Beginning with flask A, 50 cc. of a saturated solution of sodium carbonate to which has been added 5 grams of sodium chloride, is now introduced, the stopper of the flask is immediately inserted, and connection is made by means of a short piece of rubber tubing between the outlet tube of the flask and the inlet of the aspiration tube 1, which contains the standard acid. A slow current of air is now started through the solutions of the first unit of the system, comprising flask A of the diagram of fig. 1 and its corresponding absorption tube 1. In a similar manner, the second unit of the system is now prepared and joined to the first by means of a short rubber tube con-

nection, as indicated in the diagram. Each unit is thus prepared and joined in the series in regular order until the battery is completed. Aspiration is allowed to proceed slowly at first, then gradually increased to the desired rate, and allowed to continue rather vigorously until all the ammonia is removed. In the work here reported, aspiration was always allowed to proceed during a period of not less than twelve hours.

Aspiration is accomplished by the use of an ordinary filter pump attached to a faucet, but it was found that a more uniform current of air can be obtained by interposing a large carboy between the apparatus and the suction pump. This makes it possible to attach also several batteries of flasks and absorption tubes to the filter pump at this point, in case a large number of samples are to be determined at the same time. The amount of air passing through the system can be regulated by opening or closing the screw clamp at Y.

Flask X contains a Bunsen valve to prevent the backward rush of air, in case something should go wrong with the suction pump. Care must be taken to prevent this sucking back, and if for any reason it is desired to disconnect a flask in the system, the aspiration is slowed down and a few screw clamps placed on the rubber connections just back of the point of detachment. The air entering the system is freed from ammonia by drawing it through a 10 per cent. acid solution.

Less trouble from frothing occurs when a flask is used which increases in diameter just above the surface of the liquid, and when the oil is blown through a glass tube with the end so constricted that the oil can be spread on the sides of the flask and over the surface of the liquid. Excessive frothing may be prevented by the addition of a drop of capryl alcohol.

After aspiration has continued during the required period of time, the system is disconnected, starting with the flask and tube farthest removed from the suction pump and working forward. It is best to have a small current of air passing through the apparatus while this is being done. The standard acid in the absorption tubes is now titrated and the nitrogen present is calculated as ammonia. To the ammonia-free extract in each flask is now added a sufficient amount of sodium hydroxide to make this approximately one-eighth normal (in these tests, 5 cc. of 6 per cent. sodium hydroxide was added), and 2 to 2.5 grams of Devarda's alloy which has been previously ground fine enough to pass a 100 mesh sieve. The flasks are immediately connected, as before described, with a new series of absorption tubes containing the standard acid, and aspiration is again continued for a period of from twelve to fourteen hours. The standard acid in the tubes is titrated against standard sodium hydroxide and the nitrates in the sample are calculated. Whenever a 100 cc. sample of the plant extract is

used, it is found best to add the sodium salts in the solid state, in order to keep down the volume. In such cases, 10 grams of sodium carbonate and 6 grams of sodium chloride per hundred cc. of plant extract is used.

In testing out the method, duplicate samples of solutions containing nitrogen, or of plant extracts, were sometimes employed in double batteries of flasks and tubes, the sodium salts being added to the samples in both series, but the sodium hydroxide and the Devarda's alloy were added to the samples of only one of the two series, and both were thoroughly aspirated. The difference in the titration values of the standard acid in corresponding tubes of the duplicate series here represented the nitrates in the samples.

The standard deviations and probable errors were calculated, in all cases, according to the following formulas:

$$\text{Standard deviation } (\sigma) = \sqrt{\frac{\Sigma(F \cdot D^2)}{N}}$$

$$\text{Probable error } (E_{m.}) = \frac{\pm 0.6745 \cdot \sigma}{\sqrt{N}}$$

Experimental

In testing out experimentally the accuracy of the method here described, the first step was to determine whether nitrate nitrogen could be recovered from an aqueous solution of a pure potassium nitrate. These tests were carried out in accordance with the method described. In one series fifteen samples were used, each consisting of 50 cc. of the nitrate solution. Each sample contained five mg. of nitrogen. The second series was like the first except that each 50 cc. sample contained 10 mg. of nitrogen. The results are recorded in table I.

It will be observed from table I that the amount of nitrogen recovered from each sample corresponds very well with that of the original sample. The results of all the determinations are well within experimental error with the exception of no. 6, which is approxiamtely 0.2 mg. too high. This is equivalent to only 0.28 cc. of twentieth normal acid.

The method was then tested to determine whether both ammonium nitrogen and nitrate nitrogen could be accurately determined in a mixed solution of inorganic salts. The following salts were used:

Ferrous ammonium sulphate	2.800	grams
Potassium nitrate	2.886	"
Magnesium sulphate	1.167	"
Potassium phosphate (monobasic)	0.6047	"

The salts were dissolved separately, the solutions mixed and then made up to two liters. Eight 50 cc. aliquots were taken for analysis. Each sam-

TABLE I

RECOVERY OF NITROGEN FROM A POTASSIUM NITRATE SOLUTION

Series I			Series II		
Sample number	Nitrogen in sample	Nitrogen recovered	Sample number	Nitrogen in sample	Nitrogen recovered
1	5.00 mg.	4.99 mg.	16	10.0 mg.	9.94 mg.
2	"	4.97	17	"	9.95
3	"	5.00	18	"	9.95
4	"	4.99	19	"	9.96
5	"	4.97	20	"	9.97
6	"	5.20	21	"	10.00
7	"	5.10	22	"	10.02
8	"	5.04	23	"	9.99
9	"	5.04	24	"	9.97
10	"	5.02	25	"	9.99
11	"	5.04			
12	"	5.00			
13	"	4.97			
14	"	4.94			
15	"	5.01			
Mean $= 5.02 \pm 0.01$ mg. $\sigma = 0.06$ mg.			Mean $= 9.97 \pm 0.005$ mg. $\sigma = 0.024$ mg.		

TABLE II

RECOVERY OF AMMONIA AND NITRATE NITROGEN FROM A MIXED SALT SOLUTION

Nitrogen as ammonia in sample	Nitrogen recovered	Nitrogen as nitrates in sample	Nitrogen recovered	Total nitrogen in sample	Total nitrogen recovered
mg.	mg.	mg.	mg.	mg.	mg.
5.00	5.04	10.00	10.19	15.00	15.23
"	5.02	"	10.27	"	15.31
"	5.05	"	10.12	"	15.15
"	5.04	"	10.27	"	15.31
"	5.04	"	10.27	"	15.30
"	5.02	"	10.22	"	15.25
"	5.01	"	10.18	"	15.22
"	5.00	"	10.21	"	15.15
Mean $= 5.02 \pm 0.004$ mg. $\sigma = 0.018$ mg.		Mean $= 10.21 \pm 0.012$ mg. $\sigma = 0.05$ mg.		Mean $= 15.21 \pm 0.014$ mg. $\sigma = 0.06$ mg.	

ple, therefore, contained 5 mg. of nitrogen as ammonia and 10 mg. as nitrate, by calculation. The results of the determinations are given in table II. The ammonia recoveries are in very close agreement with the calculated values but the nitrate recoveries are somewhat high. This mixed salt solution was checked for nitrates by other methods, and the results obtained were in quite close agreement with those in table II, indicating that nitrogen as nitrate other than that in the potassium salt was present in the mixed salt solution, which was not accounted for in the theoretical calculation.

Logically the next step in testing out the method was to make quantitative determinations of nitrate nitrogen in the presence of organic matter. An extract was made from 100 grams of Paper White narcissus bulbs, containing no nitrate nitrogen (as shown by the diphenylamine test on an aliquot of the tissue dried at 80° C. and ground to an impalpable dust). The extract was prepared by grinding the tissue and extracting with two liters of water. This extract was then reduced in volume by evaporation to 500 cc., and ten 50 cc. aliquots were taken for analysis. Ammonia nitrogen was determined on the first four samples, then 20 mg. of nitrogen in the form of potassium nitrate was added to each of the ten samples. The system was aspirated for 14 hours and nitrate recoveries were determined. Since ammonia as well as nitrate nitrogen is recovered by this method, it was necessary in the case of the last 6 samples to subtract the previously determined ammonia nitrogen from the total recovery in order to obtain the nitrate nitrogen added to the sample. The nitrate nitrogen and the ammonia nitrogen recovered from the extract are given in table III.

These analyses show that nitrates can be recovered quantitatively in the presence of a plant extract. It is also evident from the mean recovery (20.05 ± 0.015 mg.) and the standard deviation (0.072 mg.) that no nitrogen was obtained from the organic fractions, even though each sample contained approximately 40 mg. of nitrate-free soluble nitrogen, according to the analyses of the organic fractions of the extracts of the same plant material, presented in table IV through the courtesy of Dr. G. T. Nightingale and Dr. W. R. Robbins. This table appears also in Bulletin no. 472 of the New Jersey Agricultural Experiment Station.

The results of the analyses as given in table III thus clearly indicate that any nitrates present in the plant extract may be completely reduced to ammonia in an eighth normal alkaline solution, by the method here employed, without any danger of the liberation of nitrogen from the organic fractions.

To determine still further the action, if any, of a cold eighth normal alkaline solution on the organic fractions in a plant extract during the process of the reduction of nitrates to ammonia, the following experiment was

TABLE III

RECOVERY OF NITROGEN FROM POTASSIUM NITRATE WHEN ADDED TO A NITRATE-FREE EXTRACT OF PAPER WHITE NARCISSUS BULBS

SAMPLE NUMBER	NITROGEN AS AMMONIA IN THE EXTRACT	SAMPLE NUMBER	NITROGEN AS NITRATES ADDED TO SAMPLE	NITROGEN RECOVERED AS NITRATES
	mg.		mg.	mg.
1	0.44	1	20.00	20.05
2	0.41	2	"	19.97
3	0.42	3	"	20.01
4	0.41	4	"	19.97
		5	"	19.97
		6	"	20.09
		7	"	20.18
		8	"	20.16
		9	"	20.07
		10	"	20.07
Mean = 0.42 ± 0.004 mg. σ = 0.012 mg.			Mean = 20.05 ± 0.015 mg. σ = 0.072 mg.	

TABLE IV

NITROGEN FRACTIONS OF PAPER WHITE NARCISSUS BULBS AS PERCENTAGE OF DRY WEIGHT

FRACTION	CENTERS	STORAGE TISSUE*
Total nitrate-free nitrogen	2.38	1.59
Protein nitrogen	1.59	1.25
Nitrate-free soluble nitrogen	0.79	0.36
Proteose nitrogen	0.22	0.15
Basic nitrogen	0.23	0.05
Amide nitrogen	0.13	0.07
Amino nitrogen	0.086	0.04
Ammonia nitrogen	0.008	0.003
Humin nitrogen	0.006	0.004
Other nitrogen	0.110	0.041

* Outside portions of bulbs consist of storage tissue, pure white in color. Centers are composed of immature cells and the tissue is yellow. Both regions are well defined and separated by a sharp line of demarcation.

carried out. Two series of oat plants were grown in culture solutions and were harvested just before coming into head. The plants of series I were

grown in a solution having a relatively high ammonium and a relatively low nitrate content. Ninety grams of green tissue were extracted in the usual manner. Series II was grown in a culture solution with a relatively high nitrate and low ammonium content. Eighty-three grams of the green plant tissue were extracted. Fifty cc. samples of these extracts were used in the analyses. To all samples were then added the sodium chloride and sodium carbonate, as previously described for the ammonia determination. To the even numbered flasks in the battery was added also sufficient sodium hydroxide to bring the extract to approximately an eighth normal solution. That is, the extracts in the even numbered flasks were treated as for nitrate determinations except that the Devarda's alloy was omitted. The extracts were now aspirated for a period of from 12–14 hours and the ammonia determined. The results of the tests are presented in table V, which is self-explanatory.

TABLE V

EFFECT OF EIGHTH-NORMAL SODIUM HYDROXIDE UPON THE ORGANIC FRACTIONS OF THE EXTRACT OF OAT PLANTS

	Extract plus sodium carbonate and sodium chloride as in an ammonia determination		Extract plus sodium salts and sodium hydroxide as in a nitrate determination with alloy omitted	
Series I 90 grams green tissue extracted	Sample number	Nitrogen as ammonia in sample	Sample number	Nitrogen as ammonia plus organic N due to hydrolysis
Oats grown in a solution with high ammonium and low nitrate content		mg.		mg.
	1	2.22	2	2.26
	3	2.24	4	2.28
	5	2.18	6	2.29
	7	2.22	8	2.29
	9	2.26		
	Mean = 2.22 ± 0.008 mg. σ = 0.027 mg.		Mean = 2.28 ± 0.04 mg. σ = 0.012 mg.	
Series II 83 grams green tissue extracted				
Oats grown in a solution with high nitrate and low ammonium content		mg.		mg.
	1	0.41	2	0.46
	3	0.41	4	0.42
	5	0.41	6	0.45
	7	0.39	8	0.40
	9	0.36		
	Mean = 0.40 ± 0.006 mg. σ = 0.020 mg.		Mean = 0.43 ± 0.008 mg. σ = 0.024 mg.	

From the data of table V it will be observed that the nitrogen recovered from the extracts in the even numbered flasks of series I is just slightly higher, in every case, than that recovered from the extracts in the odd numbered flasks. This indicates that the eighth normal hydroxide does have a low hydrolyzing influence upon the organic fractions of the extract; but this is so slight as to be practically negligible. The 50-cc. samples of the extracts in this series each contained, as determined by analyses, about 150 mg. of organic nitrogen. Of this amount, only 0.06 mg., liberated in the process of analysis, can be attributed to the hydrolyzing effect of the eighth normal hydroxide. This is well within the limits of experimental error.

In series II, the ammonia content of the extracts was much lower than in those of series I. In this series, as in series I, the nitrogen recovered which can be attributed to the hydrolyzing effect of the eighth normal hydroxide is practically negligible, being only 0.03 mg. per sample, although the organic nitrogen present in each sample was approximately 125 mg., as determined by analysis.

It should be pointed out that the omission of the Devarda's alloy in these tests might be a serious objection, since it is conceivable that the alloy might have some influence in liberating nitrogen from the organic fractions by increasing the catalytic action of the alkali. If this were probable, such consistent nitrate recoveries as those indicated in table III could scarcely have been obtained. However, to meet such an objection, assuming that it is a valid one, the following experiment was conducted.

Four ammonia determinations and 4 nitrate determinations were made on the nitrate-free extract obtained in the usual manner from 150 grams of Paper White narcissus bulbs. Since no nitrates were present in the extract, only the free ammonia should be recovered, on the assumption that the alkali and Devarda's alloy added to the extract in the nitrate determination can liberate no nitrogen from the organic fractions. The analyses were carried out in the manner previously described, and the data are presented in table VI.

The data of table VI again show slightly higher nitrogen recovery by the nitrate method than by the method for ammonia. The difference by the two methods, however, is only 0.05 mg. for each sample, this being equivalent to but 0.07 cc. of a twentieth normal acid. These tests, in connection with those of the preceding experiment, indicate quite clearly that the presence of the Devarda's alloy has no influence whatever in liberating nitrogen from the organic fractions of these plant extracts.

It should be noted, however, that in all the analyses of plant extracts reported here, there is always a very slightly but consistently higher nitrogen recovery when eighth normal sodium hydroxide is added to the extract

TABLE VI

EFFECT OF THE NITRATE METHOD UPON THE ORGANIC NITROGEN IN THE NITRATE-FREE EXTRACT OF PAPER WHITE NARCISSUS BULBS

EACH SAMPLE CONTAINS ONE-TENTH OF THE EXTRACT FROM 150 GRAMS OF BULBS

Nitrogen by ammonia method		Nitrogen by nitrate method	
Sample number	Nitrogen recovered as ammonia	Sample number	Nitrogen recovered as ammonia
	mg.		mg.
1	0.73	5	0.83
2	0.71	6	0.75
3	0.69	7	0.70
4	0.70	8	0.70
Mean = 0.71 ± 0.005 mg. σ = 0.016 mg.		Mean = 0.74 ± 0.018 mg. σ = 0.017 mg.	

than when it is omitted, although the difference in the corresponding values obtained by the two methods is well within experimental error. This slight but consistent difference is undoubtedly due either to incomplete recovery of nitrogen as ammonia in the absence of the eighth normal sodium hydroxide, or to slight hydrolysis of the organic fractions in its presence. The organic fractions of some biological materials may, of course, undergo alkaline hydrolysis more readily than do those of the plant extracts here employed. It is well, in any case, to test the action of the alkali on the material in question when making nitrate determinations by the method here described, as was done in the case of the extracts from the oat plants reported in table V.

RANKER (8), in showing the inaccuracies of the Devarda method for determining nitrates in the presence of organic matter, found, of twenty-four nitrate-free materials tested, that glycine and urea were among those giving the highest yield of ammonia. These two compounds, as well as arginine, were therefore subjected to a nitrate analysis by the method here described. The sodium carbonate and sodium chloride solutions were added to each of the 50-cc. samples, but no ammonia was recovered from any of the materials after twelve hours of vigorous aspiration. The solutions were then made eighth normal with sodium hydroxide and one gram of alloy was added to each sample as in a nitrate determination, and aspiration was continued for 12 hours. The results obtained are given in table VII.

Approximately 1.8 per cent. of the nitrogen in arginine was liberated even by this method. This hydrolysis might be expected, since, according

TABLE VII

ACTION OF THE NITRATE METHOD ON ARGININE, GLYCINE AND UREA

	ARGININE 32 MG. NITROGEN IN EACH SAMPLE	GLYCINE 46.6 MG. NITROGEN IN EACH SAMPLE	UREA 23.3 MG. NITROGEN IN EACH SAMPLE
SAMPLE NUMBER	NITROGEN OBTAINED	NITROGEN OBTAINED	NITROGEN OBTAINED
	mg.	mg.	mg.
1	0.63	0.26	0.00
2	0.63	0.21	0.03
3	0.59	0.41	0.00
4	0.63	0.26	0.07
5	0.59	0.37	0.00
6	0.60	0.23	0.09
7	0.56	0.37	
8	0.56	0.26	
	Mean = 0.60 ± 0.007 mg. σ = 0.028 mg.	Mean = 0.31 ± 0.014 mg. σ = 0.061 mg.	Mean = 0.03 ± 0.010 mg. σ = 0.036 mg.

to PLIMMER (**6**), arginine yields ammonia by alkaline hydrolysis more readily perhaps than any other amino acid. The quantitative determination of arginine is based upon the fact that one-half of its nitrogen is liberated by boiling with alkali by the VAN SLYKE method (**10**).

Of the 46.6 mg. of nitrogen in each sample of the glycine solution, 0.3 mg., or 0.66 per cent., was broken down to ammonia. This is a much higher percentage than was obtained from any of the plant extracts studied and is an indication that with some biological materials it may be necessary to determine the ammonia liberated by the eighth normal alkali during the process of nitrate reduction and to deduct this from the total ammonia liberated during the process, as suggested above. As the last column of table VII indicates, only a trace of nitrogen was obtained from the 23.3 mg. contained in each sample of the urea solution.

From a consideration of the data presented in the tables of the foregoing pages, and of those obtained from many other tests of the method here described but not included in these tables, it is apparent that nitrates may be determined in plant tissues by this method with a high degree of accuracy, and it is hoped that with modifications the method may be generally useful in determining the inorganic nitrogen fractions of plant biological materials.

LABORATORY OF PLANT PHYSIOLOGY,
NEW JERSEY AGRICULTURAL EXPERIMENT STATION.

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BRIEF PAPERS

EFFECT OF SUNSHINE AND SHAPE OF FRUIT ON THE RATE OF RIPENING OF TOMATO FRUITS*

The accompanying table (table I) shows high positive correlation between the number of sunshine hours and the number of days required for tomato fruits to reach full maturity after they had started to redden. This is surprising in view of the fact that light is not considered essential for the ripening processes of the tomato fruit. (1) The fruits are in fact ripened, by many commission merchants, in darkened rooms with or without the addition of ethylene. (2) DUGGAR[1] found that heat and oxygen, but not light, were factors influencing the rate of color formation.

TABLE I

COEFFICIENT OF CORRELATION BETWEEN NUMBER OF SUNSHINE HOURS AND THE NUMBER OF DAYS REQUIRED FOR TOMATOES TO REACH FULL MATURITY AFTER THEY HAD STARTED TO REDDEN

VARIETY	NUMBER OF FRUITS INVOLVED	COEFFICIENT OF CORRELATION
Baltimore	75	0.657 ± 0.044
Marglobe	73	0.400 ± 0.066
Red Pear	255	0.555 ± 0.029
Ponderosa	42	0.680 ± 0.053

The results reported herewith were obtained in the Purdue greenhouses during the fall of 1927 and winter of 1928. Each plant was grown in a galvanized iron pot containing 40 pounds of rich loam soil. The plants were not allowed to become starved for any of the mineral elements nor to suffer from lack of moisture. The night temperature was regulated at approximately 60° F. and the day temperature at 70–75° F. The higher day temperature was maintained only on sunny days. Though the high correlations secured seem to indicate that a greater amount of sunshine may have been responsible, under the conditions of this experiment, for the more rapid ripening of the tomato fruits it is nevertheless possible that the higher temperatures may have been entirely responsible. It is, however,

* Published with the approval of the Dean of the School of Agriculture and the Director of the Agricultural Experiment Station of Purdue University.

[1] DUGGAR, B. M. Lycopersicin, the red pigment of the tomato, and the effects of conditions upon its development. Washington Univ. Studies 1: 22–45. 1913.

TABLE II

COEFFICIENT OF CORRELATION BETWEEN SHAPE AND NUMBER OF DAYS REQUIRED FROM POLLINATION TO MATURITY FOR TOMATO FRUITS

VARIETY	NUMBER OF FRUITS USED	COEFFICIENT OF CORRELATION	MEAN SHAPE OF FRUIT	NUMBER OF DAYS FROM TURNING TO RIPENESS	NUMBER OF DAYS FROM POLLINATION TO MATURITY	MEAN WEIGHT (GRAMS)
Baltimore	75	-0.130 ± 0.077	1.41 ± 0.002	4.93 ± 0.12	66.3 ± 0.70	142.3 ± 4.2
Marglobe	73	0.002 ± 0.079	1.32 ± 0.009	5.42 ± 0.13	64.6 ± 0.52	153.8 ± 3.96
Red Pear	255	0.149 ± 0.041	0.76 ± 0.003	5.88 ± 0.09	56.0 ± 0.17	11.8 ± 0.11
Ponderosa	42	-0.224 ± 0.098	1.50 ± 0.013	7.00 ± 0.21	63.7 ± 0.56	226.2 ± 6.96

plausible that the greater amount of light favored the development of the red color of the tomatoes in this experiment as they were ripened on vines which were grown during the fall and winter under inadequate light conditions. DUGGAR did not test the effect of light on the rate of ripening of fruits that were still attached to the vines and although most of the fruits were grown in the greenhouse he does not state that light may have been a limiting factor for growth. Moreover the temperatures, 60–75° F., maintained during the Purdue tests were considered by DUGGAR as satisfactory for the formation of the red color of tomatoes.

There was no significant correlation between the shape

$$\left(\text{shape} = \frac{\text{equatorial diameter}}{\text{polar diameter}}\right)$$

of fruit and the rate of ripening as is shown in table II, although the tendency is for the rapid ripening of ovate fruits (Red Pear) and for the slower ripening of oblate fruits such as Baltimore and Ponderosa.

There seems to be some correlation between the weight of fruit and the number of days required for maturity although the Baltimore is a notable exception and the other data are not altogether significant.

The ovate fruit shape also seems to be correlated with the smaller fruits (*i.e.*, those that weigh less) although the Marglobe fruits prove an exception to this rule. This agrees with the results secured by LINDSTROM[2] in working with hybrids of ovate and oblate varieties of fruit.—C. L. BAKER and H. D. BROWN, *Purdue University.*

TWO PLANT MATERIAL DRIERS

(WITH FOUR FIGURES)

In the preparation of plant material for the study of enzyme content it sometimes becomes necessary to hasten the process of drying if one wishes to retain the enzymes as they are in the living plant. The drawings on another page show a drier adapted to the use of heat, and another to the use of sulphuric acid. In both, the "dead" air around the material is obviated, in one case by the use of a fan, and in the other by the movement of the material in a rotating basket.

In the first (fig. 1), warm air is forced directly over the material, and no "dead" air is allowed to retard the drying. Most plant leaves dry within four hours at a temperature of 42° C. Quiet unheated air requires about 24 to 36 hours for a less satisfactory drying. The walls are of beaver board one half inch apart. The cross partitions are of copper wire fly-screen. A longitudinal partition separates them at the middle, making six compartments in all.

2 LINDSTROM, E. W. The inheritance of ovate and related shapes of tomato fruits. Jour. Agr. Res. 34: 961–985. 1927.

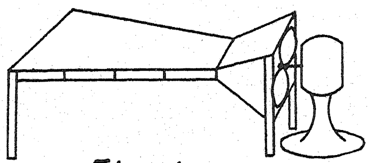

Fig. 1.

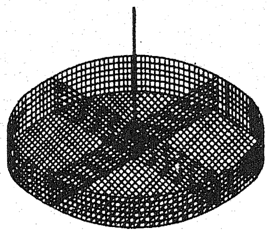

Fig. 3.

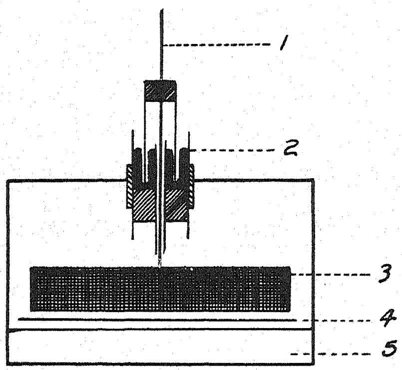

Fig. 2.

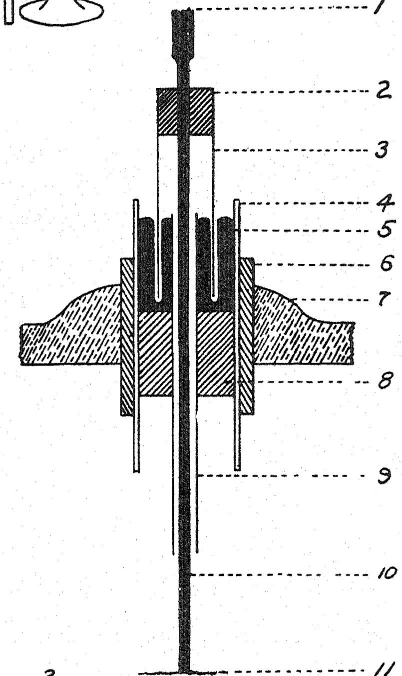

Fig. 4

FIG. 1. Hot air plant drier.

FIG. 2. Section of sulphuric acid plant drier. 1. Iron shaft. 2. Mercury seal. 3. Rotating basket. 4. Hardware cloth screen. 5. Sulphuric acid.

FIG. 3. Detail of rotating basket. The top circular piece is not shown.

FIG. 4. Detail of mercury seal. 1. Attachment of shaft to motor. 2. Cork stopper through which the shaft is tightly fitted. 3. Glass tube rotating with the shaft. 4. Larger glass tube. 5. Mercury. 6. Cork with hole to accommodate glass tube "4." 7. Lid of desiccator. 8. Cork. 9. Glass tube. It should extend above mercury. 10. Shaft. 11. Attachment of shaft to basket.

The heating element is made of three feet of coiled wire similar to that used in hot plates. It is wound around a transite piece 2 x 8 inches. The fan is 9 inches in diameter, and rotates within the hood.

The "cold" drier (fig. 2) is not so rapid but may be employed at or much below room temperature. Its efficiency lies in the moving basket. We have found the electric motor stirrer most suitable for power. It is attached directly to the shaft and rotates at a speed of 70 r. p. m. The basket (fig. 3), has four compartments, and is made of one-half inch mesh hardware cloth. Two circular pieces held two inches apart by a cross of the same material are closed in by an outside band. If the cross-pieces are cut so as to allow the wires to extend on each side, and these used to bind them to the top and bottom of the basket, a solid construction is assured. A portion of the top is cut out above each compartment, a shaft soldered in the center and it is ready for service. A cloth lining may be needed when some material of smaller pieces is being dried.

Fig. 4 shows a detail of the mercury seal. If warm paraffin is poured into the mercury moat and out again it will be made mercury-tight.

The time of drying is reduced about two-thirds over that of non-moving materials above sulphuric acid.

Experiments are under way to test the relative merits of these two driers for drying plant materials for use in the study of plant enzymes.—A. G. Wood, *Grove City College, Grove City, Pa.*

NOTES

Summer meeting of the American Society of Plant Physiologists.—At the invitation of the Purdue Section, the summer meeting of the Society was held at Purdue University, Lafayette, Indiana, September 4 and 5. About fifty were in attendance, mostly from the states of Ohio, Michigan, Illinois, Indiana, and Wisconsin.

On September 4 a program of short papers was given by members of the Purdue staff, explaining investigations and field plot work. Mr. J. F. TROST discussed the response of corn hybrids to fertilizers. Mr. L. P. MILLER gave the results of his studies on the effect of manganese deficiency in sand culture. Dr. E. B. MAINS reviewed the results of studies concerning physiologic specialization in the rusts. Mr. D. K. DOAK gave the results of his investigations concerning the effect of mineral nutrition on the rust reaction of wheat. Dr. J. H. MACGILLIVRAY summarized the results of his studies concerning tomato quality. Prof. L. P. CULLINAN and Mr. J. L. SULLIVAN gave the results of their studies concerning the nutrition of apple trees. Prof. H. P. BREWER described methods for the purification of the virus of tomato mosaic. Mr. L. M. BUSHNELL described the dominant soil types in the vicinity of Lafayette. Prof. S. D. CONNER outlined the agronomy field plot experiments. Following the dinner, which was held at Lincoln Lodge, Dr. C. A. SHULL discussed the present status of the journal, PLANT PHYSIOLOGY, and plans for its future development.

September 5 was spent in field trips. In the morning the Soils and Crops experimental farm east of Lafayette, and the Animal Husbandry farm north of West Lafayette were visited, and breeding and fertility experiments with corn were studied. Following a lunch at the Fowler Hotel, Prof. E. J. KRAUS, President of the Society, discussed the relationship of plant physiology to applied botany. In the afternoon the orchards of the Horticultural Department west of Lafayette were visited, and pruning and fertility experiments were studied. Taken as a whole, the meeting was very successful, and the Purdue Section is to be congratulated on the manner in which the arrangements were carried through.

The Fifth Annual Meeting.—The annual meeting to be held at New York in December, 1928, is the fifth annual meeting of the Society. As this note is being prepared, the program arrangements are reaching completion. Members who can attend these meetings should offer their best contributions to the Program Committee. The main outlines of the program indicate a fine meeting, with two excellent symposia, one on Cell

Physiology, and the other on Radiant Energy Relations of Plants. These are held jointly with the Physiological Section of the Botanical Society of America.

The headquarters hotel has been announced as the Lincoln Hotel. Every member who can do so should attend the New York meeting. It is one of the quadrennial meetings of the American Association for the Advancement of Science, and offers an opportunity for scientists of all fields of work to consolidate their forces for the promotion of greater freedom and greater privileges for the leaders of scientific progress.

Those who attend the meeting should plan to be present at the annual dinner for all plant physiologists. The announcement of the first STEPHEN HALES prize award, and the award of the third CHARLES REID BARNES Life Membership should make this a memorable occasion.

The New Constitution.—Copies of the new constitution have been sent out to the members of the Society by the Secretary. The growing activities of the Society have made necessary a more detailed instrument than the one under which the Society has been operating. The provisions should be examined critically by all members, to uncover any defects which may still be present. The instrument will be more difficult to change after adoption than before, and every effort should be made to bring it to perfection before the vote is taken.

Size of Manuscripts.—The matter of size of manuscripts suitable for publication in PLANT PHYSIOLOGY has been mentioned by several members. The value of a manuscript is obviously not determined by its length, and the editorial committee has not set any definite limit upon the size of papers that may be published. Some short papers are much more valuable than other longer ones, and the editors are far more concerned about the quality than about the size of papers submitted for examination. However, there are limitations to the volume of material which can be printed. PLANT PHYSIOLOGY can use enough manuscript each year to print about 500 pages per volume without deficit at its present rate of income. That means that we could use 16–18 papers, each printing up thirty pages, within a year. But if the papers run 10 to 15 pages, we can use 40 to 50 in a year. Many a long paper can be improved by condensation, and elimination of material with which everybody is familiar. There is often a tendency to restate the obvious, and to spin out introductions to unnecessary length. The main need is for papers that are well organized, full of new information, thoroughly digested, and clearly written. The editorial board will probably continue to accept papers of almost any length that measure up to their

standard of value. But contributors are asked to keep in mind the limitation of size on the annual volume of publication, and to make papers as concise as good presentation permits.

Carbon Copies of Manuscripts.—It is probable that most of the authors who submit manuscripts to PLANT PHYSIOLOGY retain carbon copies of their papers. This is a desirable practice. So far, there has been no loss of a manuscript in the mails, but in passing around the editorial board each paper makes a journey of six thousand miles. Each paper is mailed five times, and there is always some risk of loss. The seriousness of such a loss will be minimized if the author has a carbon copy in reserve. It also facilitates correspondence between editor and author about changes in the paper, if they seem desirable, and does away with mailing of manuscripts with proofs.

Costs of Publication.—In order that the members of the American Society of Plant Physiologists may be informed as to the costs of publication of the official journal, the following figures are presented, showing the various items which make up the cost of a single issue of PLANT PHYSIOLOGY. The April number is used as an example:

10 pt. text	$372.78
8 pt. text	48.84
Tabular	99.24
Blanks	8.70
Engraving	105.74
Changes	22.50
Stencils	.48
Postage	11.13
Cover	11.17
Wrapper and mailing	3.50
Reprints	8.30
Total	692.38

The two items which need most careful attention are those for tabulated data, and for the illustrations.

Cost of Reprints.—The high cost of reprints is an evil which we have not been able to abate. Authors have a right to know the basis upon which charges for this service are calculated, so that they can estimate approximately the cost of the reprints ordered. We are presenting, therefore, the rates now charged by the Science Press Printing Company for the printing of separates for authors.

	1 page	4 pages	8 pages	16 pages	32 pages
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Portrait of Timiriazeff.—The portrait of TIMIRIAZEFF in the April number is only the first of a series of portraits of famous plant physiologists to be published in PLANT PHYSIOLOGY. Believing that there are a number of plant physiologists who would like to place these portraits in frames and hang them upon the walls of their class rooms and laboratories, a few copies were made without the plate number and journal name. While they last, copies may be obtained from the editor of PLANT PHYSIOLOGY for twelve cents. As the number printed was not large, those who desire copies should send for them early.

Joseph Tooker Rosa.—It is with much regret that we have to record the death of Dr. JOSEPH T. ROSA, one of the staunch supporters of the Society, who died suddenly at his home in Davis, California, on August 8, 1928. Although but 33 years old, he had contributed many valuable papers on the management of truck plants, such as potatoes, sweet potatoes, tomatoes, melons, and spinach. A South Carolinian by birth, he was reared in a region where truck cropping was an important industry. His early contacts with the problems of production and storage of vegetable crops no doubt gave him the incentive to his scientific career. He received his first degree from Clemson at the age of 20, and his M.S. two years later from Iowa State College, while occupying a position as instructor in crop plants. During the succeeding five years he was a member of the horticultural staff at the University of Missouri, where he received his Ph.D. in 1922. During the last six years he has been associate professor of truck crops at the University of California, and associate plant breeder in the California Agricultural Experiment Station, at Davis, California. He will be greatly missed by his many friends.

Harris Miller Benedict.—The tragic death of Dr. H. M. BENEDICT, Professor of Botany at the University of Cincinnati, in an automobile col-

lision on October 17, while driving from his home to the University, came as a shock to all of his friends. Dr. BENEDICT has been a member of the American Society of Plant Physiologists for several years, and was much interested in the growth and development of the organization. Recently he had been appointed as one of the representatives of the Society on the Council of the American Association for the Advancement of Science. He was also a generous contributor to the STEPHEN HALES prize fund.

Professor BENEDICT was born in Illinois, received his collegiate training at Doane College, and at the University of Nebraska, taking his M.S. degree at the latter institution in 1897. A few years later he became a member of the faculty of the University of Cincinnati, and has been the leader of the development of the botany department there for over a quarter of a century. The department grew strong under his leadership, and the students and faculty members who worked with him caught the inspiring spirit of his life and work. They loved him for his fine sympathy and understanding, and for the rare quality of his friendship. And they loved botany, for he presented it as a great humanistic science.

While he received his Ph.D. degree from Cornell in 1914 in plant physiology, he was interested in a much wider field. He loved birds, knew them intimately, and was the originator and director of the Emery Bird Reserve, the first municipal reserve of this nature to be established. He was also interested in botanical gardens, particularly in an Ohio botanical garden, and as director of the Ohio Botanical Garden Society, was working for the establishment of research botanical gardens to solve the plant problems of industry.

A man of fine spirit, courteous, kind, sympathetic, enthusiastic, it will be difficult to replace him; his loss will be felt keenly among American botanists.

International Critical Tables.—The fourth volume of International Critical Tables has come from the press of the McGraw-Hill Co. It contains phase equilibrium data, including those for triple points, transition points, and melting points at ordinary and low temperatures; the effects of pressure upon the melting and transition points; volume changes on melting and transition; directly measured compressibility and thermal expansion differences. There is also a section dealing with the properties of silica, and a valuable freezing point-solubility table. The following sections present data on the solubility of salts, strong acids, and bases in water; and the solubility of organic substances and weak electrolytes in water. Data on the freezing point lowering of aqueous solutions of inorganic strong electrolytes and all salts are given. These data occupy the

first 429 pages of the volume. A brief section considers the data on osmotic pressure of cane sugar solutions, colloids, etc.

The later pages of the volume are devoted to the properties of surfaces, (surface tension, surface energy and related problems), tensile strength and angle of contact, methods of measuring surface tension, and tables for the interfacial tensions of solid/liquid and liquid/liquid interfaces. An important section by T. FRASER YOUNG and WILLIAM D. HARKINS presents surface tension data for certain pure liquids between 0° and 360° C., and for all types of solutions at all temperatures.

A temporary index to volumes I–IV inclusive has been prepared, and a sheet of errata for the first 3 volumes comes along with volume IV. The set lacks only one volume of completion up to the end of 1923. Annual volumes beginning with 1924 will keep the information up to date. Every research laboratory needs this excellent reference work.

Handbook of Chemistry and Physics.—The thirteenth edition of this handbook, published by the Chemical Rubber Publishing Co., Cleveland, Ohio, has just appeared. It contains about 100 pages of new material, and various improvements in the tables. The regular price is $5.00, but students are permitted to obtain it at half price. This handbook has been a valuable aid to students during the last 15 years, and deserves its popularity.

A Textbook of General Botany.—The Wisconsin textbook of general botany appears in a revised edition, with numerous small changes, but without radical departure from the presentation in the previous edition. It has many features to commend it, and will probably continue to be used rather widely. It is a Macmillan publication, and is listed at $3.75.

A Laboratory Manual of Botany.—This laboratory outline of general botany by EMMA L. FISK and RUTH M. ADDOMS, is designed to accompany the Wisconsin textbook of botany. There are 36 exercises, 13 of which are devoted to a general study of the seed plants as organisms, and 23 to a survey of the plant kingdom. At the close of the outline there are some helpful suggestions for the selection and preparation of materials used in the laboratory. This manual will be welcomed, not only by those who use the general textbook of botany which it parallels, but by those many teachers who want a workable outline for the laboratory work in botany. It is reasonably priced, $1.00, and is a Macmillan publication.

Elements of Botany.—To meet the need for a text covering an introduction to botany in a single semester, R. M. HOLMAN and W. W. ROBBINS

have abridged their textbook of general botany. The general arrangement is much the same as in the larger volume, and also much like the Wisconsin text in approach and arrangement of chapters. The treatment is brief, but 15 chapters, which presents the essentials of a beginning course in an attractive manner. The book is published by Wiley and Sons, at $2.75, and is well adapted to the one semester introduction.

Statistical Methods for Research Workers.—A second edition of R. A. FISHER's monograph on statistical methods has appeared from the press of Oliver and Boyd, Edinburgh and London. Professor FISHER is chief statistician of the Rothamsted Experimental Station, and his experience in handling small sample problems is placed at the service of those who need statistics in the interpretation of their results. Plant physiologists often find it advantageous or even necessary to use statistical methods in analyzing the variable results of experiments. FISHER's work is a very valuable aid in the analysis of variability, in testing the significance of means, and in determining coefficients of correlation. The 6 tables included in the text are reproduced at the end of the book in folding copies, which may be detached and mounted for convenient consultation. The price of the book is 15 shillings net. Orders should be sent to Oliver and Boyd, Tweeddale Court, Edinburgh.

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